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(54) Title: PHAGOCYTOTIC ASSAY METHOD (57) Abstract <p>The invention provides assay methods for determining whether a compound is an enhancer or an inhibitor of a signal transduction pathway which promotes phagocytosis of apoptotic cells. The methods involve exposing a phagocytic cell to apoptotic cells, optionally transfected with a reporter gene, and measuring the extent of phagocytosis in the presence or absence of the test compound. Expression vectors are provided to transfect mammalian cells with DNA sequences which when expressed influence the rate of phagocytosis of apoptotic cells such as the human homologue of the <i>C. elegans</i> ced-6 gene.</p>			
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PHAGOCYTIC ASSAY METHOD

5 The present invention relates to the field of
programmed cell death or apoptosis and in particular
to the phenomenon whereby apoptotic cells are rapidly
phagocytosed or engulfed by other cells.

Specifically, the invention provides assays and
materials for use therein, which measure phagocytosis
of apoptotic cells. Such assays can be used to
10 identify chemical substances which influence the
phagocytic uptake of apoptotic cells and have
potential pharmacological activity. The assays of the
invention are well adapted for medium-and high-
throughput screening using a multi-well plate format.

15 During development and maintenance of living tissues a
large number of cells undergo programmed cell death or
apoptosis. This is observed in both vertebrates and
invertebrates. For example, it has been shown that in
20 the nematode *C. elegans* 131 cells undergo programmed
cell death (Lui and Hengartner (1997) early 1997
International Worm Meeting, Abstract 371). Lysis of
the apoptotic cells is potentially harmful since their
contents may cause toxic damage to the surrounding
25 tissues. It has been observed that this harmful
effect is avoided because apoptotic cells are engulfed
and subsequently degraded by other cells. In mammals
the engulfing cells may be professional or semi-
professional phagocytes such as neutrophils or
30 macrophages or they may be neighbours of the dying
cells.

A key feature of the process of programmed cell death,
or apoptosis, is the efficiency with which the dying
35 cells are recognized and engulfed by phagocytes
(Savill, J.et. al, Immunol Today, 14:131-136, 1993.).
Apoptosis triggers a distinct sequence of events

characterized by the expression of phosphatidylserine on the cell surface, DNA fragmentation or laddering and the release of membrane-bound cell fragments called apoptotic blebs and bodies (Cohen, J. J.et. al, 5 Annu Rev Immunol, 10:267-293, 1992.; Kerr J.F.R.et. al, Br J Cancer, 26:239, 1972.). Apoptotic cells and bodies are phagocytosed via various receptors that recognize phosphatidylserine and other undefined ligands unique to the surface of apoptotic material 10 (Savill, J. S.et. al, J Clin Invest, 83:865-875, 1989.; Fadok, V. A.et. al, J Immunol, 148:2207-2216, 1992.; Savill, J.et. al, Nature, 343:170-173, 1990.). In this way, apoptotic cells, which contain potentially inflammatory factors, are rapidly cleared 15 by neighboring cells acting as semi-professional phagocytes or voracious experts of the macrophage line without inducing an inflammatory response (Fadok, V. A.et. al, J Clin Invest, 101:890-898, 1998.).

20 The process of apoptosis has been associated with a number of human diseases, including cancer, autoimmune diseases, various neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis, Huntingdon's disease and Alzheimer's disease, stroke, myocardial infarction 25 and AIDS (Thompson, CB, Science 267, pp 1456-1462). Thus, much attention has been focused on elucidating the mechanism of apoptosis and the genes controlling it with a view to developing new therapeutic strategies for these diseases.

30 Particular diseases have been associated with an impairment of phagocytosis of apoptotic bodies. Examples of such diseases include autoimmune diseases such as systemic lupus erythematosus, (Herrmann, M.et. 35 al, Arthritis Rheum, 41:1241-1250, 1998.), AIDS (Zocchi, M. R.et. al, AIDS, 11:1227-1235, 1997.), acute pulmonary infections (Cox, G.et. al, Am.J

Respir. Cell Mol. Biol., 12:232-237, 1995.) and allergy (Ying, S. et. al, Proc Assoc Am Physicians, 109:42-50, 1997.). It is clear that modulation of phagocytosis of apoptotic cells by drugs is a promising strategy for future therapies.

Phagocytosis of apoptotic cells in vertebrates has been observed to be a very complicated process and how any signal generated by the dying cell is received and transduced by the engulfing cell is not understood.

A swift engulfment of apoptotic cells is observed in the hermaphrodite *C. elegans* and this worm has provided a useful tool for study of the engulfment process. For example, six genes have been identified in *C. elegans* as effecting engulfment known as *ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7* and *ced-10*. Of these *ced-6* has been singled out by the present inventors for particular study. It is known that *ced-6* maps to chromosome III near *daf-4* in *C. elegans* (Lui and Hengartner (1997); Lui and Hengartner (1996) East Coast Worm Meeting Abstract 128). That work showed that two cosmids from this region, F56D2 and F43F12 could rescue *C. elegans* with a *ced-6* (n1813) engulfment defect. A 10 kb *Xho* I subclone from F56D2 with rescuing activity was identified as carrying the *ced-6* gene.

The present inventors have identified two human homologues of the *C. elegans* *ced-6* (*hlced-6* and *h2ced-6*), *h2ced-6* being a splice variant of *hlced-6* and thought to be a dominant negative version thereof. Both homologues have been shown to be present in the human cell-line THP-1. A surprising degree of sequence homology between *hCED-6* and *C. elegans* *CED-6* has been found. *hCED-6* has a phosphotyrosine binding domain from about amino acid position 11 to about

amino acid position 190 as shown in Figure 4 suggesting its involvement in a tyrosine kinase signal transduction pathway.

5 h1CED-6 and h2CED-6 proteins and their encoding nucleic acids are useful for carrying out assays as described herein to identify compounds which are inhibitors or enhancers of a signal transduction pathway which promotes phagocytosis of apoptotic
10 cells. In particular they are useful for identifying inhibitors or enhancers of h1CED-6 and h2CED-6 or inhibitors or enhancers of the transcription thereof. Such inhibitors or enhancers may be useful therapeutic agents in the treatment of some of the aforementioned
15 diseases.

In accordance with its first aspect the invention provides an expression vector capable of expressing h1CED-6 or h2CED-6 which vector comprises a sequence
20 of deoxynucleotides encoding the amino acid sequence of Figure 4 or Figure 5 or an amino acid sequence which differs from the amino acid sequence of Figure 4 or Figure 5 only in amino acid changes which are conservative of biological function.

25 The term "biological function" is defined herein to mean the ability to regulate or affect phagocytosis of apoptotic cells. Amino acid changes which are "conservative" are those which permit biological
30 function to be retained although it may be less than or greater than the level of biological function of the wild-type human CED-6 protein. Such conservative changes may include insertion or deletion of one or more amino acids or substitution of one or more amino
35 acids with another amino acid or acids having similar chemical characteristics. The choice of amino acids for making conservative changes will be well-known to

those skilled in the art.

5 In a preferred embodiment the expression vector is one comprising the sequence of deoxynucleotides shown from the transcription start codon to the transcription stop codon shown in Figure 2 or Figure 3 and optionally a vector comprising the sequence of deoxynucleotides shown in Figure 2 or Figure 3.

10 In a particularly preferred embodiment the expression vector of the invention comprises a sequence of nucleotides encoding a reporter gene positioned so that expression of h1CED-6 or h2CED-6 results in expression of the reporter gene. The reporter gene
15 may be positioned 3' or 5' to said h1ced-6 or h2ced-6 and may be expressed as a fusion protein with h1CED-6 or h2CED-6. Suitable reporter genes are those which express a fluorescent product such as green fluorescent protein (GFP). Other suitable reporter
20 genes are enzymes, such as β -galactosidase or luciferase, which are capable of acting on a substrate to produce a detectable product, for example a fluorescent product or luminescent product. Examples of expression vectors in accordance with the invention
25 are pGA3103 and pGA3104 which are shown in Figures 29 and 10 respectively.

30 In another preferred embodiment the expression vector of the invention expresses an epitope tag at the amino and/or carboxy terminal of the h1CED-6 or h2CED-6 protein. An example is the plasmid pBAD/HisA-h1ced-6 the DNA sequence of which is shown in Figure 17.

35 It will be understood that the expression vectors described above will comprise not only nucleic acid encoding h1CED-6 or h2CED-6 or functional variants thereof but also regulatory sequences operably linked

to said nucleic acid, such as promoter regions that are capable of effecting expression of the DNA fragments. The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner.

Regulatory elements required for expression include promoter sequences to bind RNA polymerase and to direct an appropriate level of transcription initiation and also translation initiation sequences for ribosome binding. For example, a bacterial expression vector may include a promoter such as the lac promoter and for translation initiation the Shine-Dalgarno sequence and the start codon AUG. Similarly, a eukaryotic expression vector may include a heterologous or homologous promoter for RNA polymerase II, a downstream polyadenylation signal, the start codon AUG, and a termination codon for detachment of the ribosome. Such vectors may be obtained commercially or be assembled from the sequences described by methods well known in the art. Promoter sequences which may be used in the expression vectors of the invention include Δ HSP, CMV, SV40, EF-1 α , UbC, SG, RSV, TRE/minCMV, HSV TK, 5', LTR and QBISP136 enhanced CMV.

Examples of expression vectors described herein are plasmids but may also be virus or phage vectors. Such vectors will normally possess an origin of replication and one or more selectable markers such as a gene for antibiotic resistance. It is particularly preferred that the expression vectors of the invention are suitable for transfection of mammalian cells and therefore may be provided with a selectable marker accordingly.

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In accordance with a second aspect the invention provides a mammalian cell-line transfected with any of the expression vectors described above. Methods of transfecting mammalian cells are well-known to those skilled in the art. The cell-line may be one which is capable of growing in monolayer culture or in suspension culture. Suitable cell-lines are fibroblast cell-lines or epithelial cell-lines such as COS1, BHK21, L929, pc12, CV1, SWISS3T3, HT144, IMR32, HEPG2, MDCK, MCF7, 293, Hela, A549, SW48 or G361. Primary cell-lines such as human dermal FIBs, dermal keratinocytes, leucocytes, monocytes, lymphocytes, dendritic cells or macrophages may also be used. Particularly preferred for use in the phagocytosis assays of the invention are mammalian professional or semi-professional phagocytes of which examples are mouse macrophage cell-line J774 or human monocyte cell-line THP1 which has been shown to express h1CED-6 and h2CED-6 (see Example 3 and Figure 6). Both of the above cell-lines may be referred to as monocyte cell-lines since monocytes are capable of differentiating into macrophages which is the form in which they are used for the assay of the invention.

In accordance with a third aspect the invention provides a method for determining whether a compound is an enhancer or an inhibitor of a signal transduction pathway which promotes phagocytosis of apoptotic cells which method comprises exposing mammalian cells transfected with h1ced-6 or h2ced-6 as described above to apoptotic particles and measuring the rate of phagocytic uptake of said particles by said transfected cells in the presence or absence of said compound. The test compound is preferably added prior to addition of said apoptotic particles.

Suitable apoptotic particles are cells such as

neutrophils, lymphocytes, erythrocytes, lymphocytes or dendritic cells which have been rendered apoptotic and are optionally opsonized by exposure to serum. Cells suitable for forming the apoptotic particles include the cell-lines L929 and PC12. A particularly preferred cell-line for use as an apoptotic particle is the growth factor dependent mouse cell-line Ba/F3. These may be grown in standard culture medium as described in Example 5 and can be rendered apoptotic by growth in the absence of the growth factor IL-3 for a suitable period (for example about 20 hours) prior to use. The apoptotic status of the cells can be determined using, for example, an annexin/propidium iodide labelling kit available from Boehringer Mannheim (Brussels, Belgium). Cells are considered early apoptotic if they are about 20% annexin positive and less than about 5% propidium iodide negative.

The PC 12 cell-line may be rendered apoptotic by growth in standard medium in the absence of nerve growth factor.

As an alternative to the cells described above the apoptotic particles could be a non-living material such as dye-labelled latex beads. 0.1 μ M, 1 μ M, 4 μ M and 10 μ M beads that have either an amino or carboxylate group are available from Sigma-Aldrich, Bornem, Belgium, or Molecular Probes, Eugene, USA.

In order for the assay described to be suitable for high-throughout compound screening it is preferred that the apoptotic particles bear some kind of detectable label so that it will be readily apparent that the particles have been taken up by the transfected mammalian cell and so that this can be quantified. The inventors have found that this may be easily achieved by stably transfecting the cells.

comprising the apoptotic particles with an expression vector comprising a reporter gene. A particularly suitable reporter gene encodes to be β -galactosidase which is capable of cleaving the fluorogenic substrate fluorescein di-b-D-galactopyranoide to a fluorescent compound which may be monitored using standard fluorescence detection equipment. Other fluorescent substrates are available for β -galactosidase. A plasmid pcDNA3.1/HIS/lacZ, which expresses β -galactosidase and is suitable for transfecting cells used as apoptotic particles, for example Ba/F3, is shown in Figure 11. Other suitable reporter genes are those encoding fluorescent proteins such as green fluorescent protein or proteins capable of generating a luminescent signal such as luciferase. Plasmids, pEGFP-N3 or PEGFP-C2, available from Clontech, are suitable for transfecting cells used as apoptotic particles with GFP and are shown in Figures 7, 8, 26 and 27. A plasmid "PGL Control" available from Promega is suitable for transfecting cells to be used as apoptotic particles such as Ba/F3 cells with a gene encoding luciferase. The DNA sequence of "PGL control" is shown in Figure 19.

It will be appreciated that the choice of reporter gene for the apoptotic particles may be governed by the presence of any reporter gene in the transfected mammalian cells. For example, the presence of the same reporter gene in the transected mammalian cells transfected with h1 or h2 ced-6 and the apoptotic cells is not *prima facie* desirable because of overlap of signals, although this may not always be the case.

It will further be appreciated that a wide variety of compounds can be tested to see whether they are inhibitors or enhancers of signal transduction pathways which promote phagocytosis of apoptotic

cells. The compound may be of any chemical formula, a polymer or a monomer. For example the test compound may be genomic DNA, cDNA, RNA, PNA, a protein or polypeptide, an amino acid, nucleoside or nucleotide.

5 The compound may be one of known biological or pharmacological activity, a known compound without such activity or a novel molecule such as might be present in a combinatorial library of compounds.

10 It will be appreciate that where any compound the presence of which results in no or a decreased amount of engulfment of apoptotic particles by the transfected mammalian cells, those cells must be tested for viability. The presence of viable cells
15 will confirm that lack of engulfment is due to the effect of the test compound on phagocytic activity and not just non-specific toxicity.

Furthermore, any compound identified as an inhibitor
20 or an enhancer of phagocytosis of apoptotic cells by the assay described above will be further tested to establish whether the effect is mediated through CED-6. In the case of a compound identified as an
25 enhancer of phagocytosis of apoptotic cells this can be achieved by carrying out a phagocytosis assay exactly as described above with mammalian cells which are not transfected with h1ced-6 or h2ced-6. If the
30 compound is able to induce a phenotype in the untransfected cells which is similar to the phenotype of those cells when transfected with h1ced-6 then it is an indication that the compound in question exerts its effect via CED-6 or via the CED-6 signal transduction pathway.

35 Similarly, if a compound identified in the above described assay is an inhibitor of phagocytosis of apoptotic cells it can be confirmed whether its effect

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is via CED-6 or the CED-6 signal transduction pathway by examining the phenotype of the transfected mammalian cells exposed to the compound. Reversion to a wild-type phenotype is an indication of action via CED-6 or the CED-6 signal transduction pathway.

In a fourth of its aspects the invention provides a method for determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells which method comprises the steps of:

- (1) micro-injecting into a mammalian cell a human CED-6 protein comprising the sequence of amino acids shown in Figure 4 or Figure 5 or a sequence of amino acids differing from that shown in Figure 4 or Figure 5 only in amino acid changes conservative of function, and
- (2) exposing the mammalian cell produced in step (1) to apoptotic particles and measuring the rate of phagocytic uptake of said particles by said cell in the presence and absence of said compound.

Preferably, the mammalian cell is micro-injected with a fusion protein comprising h1CED-6 or h2CED-6 and a reporter gene which may be any one of the reporter genes described above. Preferred fusion proteins are obtainable by expression from the GFP and h1ced-6 encoding sequences of the plasmids shown in Figures 9 or 28.

All of the preferred features and embodiments described above for assays with transfected mammalian cell-lines can be applied to cells micro-injected with

h1CED-6 or h2CED-6 or fusions thereof as described above.

5 In a fifth aspect the invention provides a method for determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells which method comprises the steps of:

- 10 (1) micro-injecting or transfecting into a mammalian cell a vector expressing RNA antisense to all or a portion of the sequence of nucleotides shown in Figure 2 or Figure 3;
- 15 (2) exposing the mammalian cell produced in step (1) to apoptotic particles and measuring the rate of phagocytic uptake of said particles by said cell in the presence or absence of
- 20 said compound.

Preferably, the antisense DNA comprises a sequence of nucleotides which is capable of hybridizing to a sequence of nucleotides as shown in Figure 2 or Figure

25 3 under conditions of stringency which are higher than 2xSSC; 0.1%SDS; 25°C to 50°C.

All of the preferred features and embodiments described above for assays with transfected mammalian

30 cell lines can be applied to the cell-lines injected with antisense RNA.

It will be appreciated that the transfected mammalian cells for use in the assays described above may be

35 transfected with h1ced-6 or h2ced-6. Since h2ced-6 is thought to be a dominant negative version of h1ced-6 having an opposite biological effect, transfected

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cells can be chosen depending on whether it is desired to identify compounds which are inhibitors or enhancers of apoptotic cell phagocytosis. For example cells transfected with h1ced-6 would be particularly suitable for identifying inhibitors of phagocytosis of apoptotic cells while cells transfected with h2ced-6 would be particularly suitable for identifying enhancers.

It is hereby stated that the invention also relates to any compound identified as an inhibitor or enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells as identified in accordance with any of the assay methods described herein.

The nucleotide sequences for h1ced-6 and h2ced-6 are shown in Figures 2 and 3 respectively. In addition cDNAs encoding the alternative splice h2CED-6 and the insert to reconstitute h1ced-6 from h2ced-6 have been deposited at the Belgian Coordinated Collections of Microorganisms (BCCM) at Laboratorium voor Moleculaire biologie - plasmidencollective (LMBP) B 9000, Gent, Belgium in accordance with the Budapest Treaty on 8th June 1998 and have been accorded the Accession Nos 3868 and 3869 respectively.

Sequences can be obtained in both deposits using T3 or T7 primers (either one or both can be used, they are at different sites of the actual insert). Both are commercially available from Clontech (~1227 and ~1228) and sequence is shown below

T7 primer: 5' (TAATACGACTCACTATAGGGAGA) 3'

35

T3 primer: 5' (ATTAACCCTCACTAAAGGGA) 3'

In addition to developing assays based on mammalian cells which over or under express human CED-6 protein the present inventors have identified epitopes of h1CED-6 and have generated useful antibodies thereto.

5 Therefore in a sixth aspect the invention comprises a fragment of human CED-6 protein having the amino acid sequence as shown in Figure 4 wherein said fragment includes the sequence of amino acids HRAFSRKKDKTC,
10 FLGSTEVEQPKGTE or TRNGTQPPPVPST. Antibody preparations have been prepared which comprise antibodies to one or more of the above epitopes. Such antibody preparations are obtainable by the method described in Example 6 and their specificity is
15 demonstrated by the Western Blots carried out in Example 7 (see Figures 20 to 25).

The antibodies described above may be used in a method of diagnosing a disease in a patient which is
20 associated with over or under expression of human CED-6 in phagocytic cells. Specifically, there is provided a method for diagnosing a disease associated with the over or under expression of human CED-6 protein in phagocytic cells in an individual which
25 comprises:

- (a) obtaining a sample of phagocytes from said individual;
- 30 (b) exposing said phagocytes to an antibody preparation as described above;
- (c) quantitatively measuring the presence of any immune complexes formed between said
35 antibodies and said CED-6 protein; and
- (d) comparing the amount of immune complex

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formed with that formed using phagocytes from a control individual.

- 5 The antibodies described above may be further used in assays for determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells. Specifically, there is provided a method for
- 10 determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells which method comprises:
- 15 (a) exposing a mammalian cell transfected with an expression vector as described above to the compound to be tested;
- 20 (b) exposing said mammalian cell to an antibody preparation as described above;
- 25 (c) quantitatively measuring the presence of any immune complex formed between said antibodies and protein expressed by said cells; and
- 30 (d) comparing the level of immune complex detected with the amount of immune complex detected in a mammalian cell transfected as described in step (a) which has not been exposed to said compound.

In the above described method the mammalian cell may be selected from COS1, BHK21, L929, CU1, SWISS 3T3, HT144, IMR32, HEPG2, MDCK, MCF7, 293, hela, A549, SW48

35 or G361 with COS1 cells being particularly preferred. Alternatively, the mammalian cell is a human dermal

FIB, dermal keractinocyte, leucocyte, monocyte, hyphocyte, dendritic cell or macrophage. Preferred are professional phagocytes such as mouse macrophage cell-line J774 or human monocyte cell-line THP-1.

5

Other uses for the antibodies of the inventions include purification of hCED-6 and identification of proteins interacting with CED-6 so that the signal transduction pathway can be characterised, detecting
10 over or under expression, cellular localization or post-translational modifications of hCED-6, epitope mapping and identification of active sites and pharmaceutical compositions comprising said antibodies in a suitable carrier or diluent.

15

In accordance with a seventh aspect the invention also provides a method for diagnosing a disease associated with the over- or under-expression of human CED-6 in phagocytic cells in an individual, which method
20 comprises:

- (a) obtaining a sample of phagocytes from said individual,
- (b) isolating RNA from said phagocytes,
- (c) preparing cDNA from said RNA,
- 25 (d) performing a first PCR reaction on said cDNA,
- (e) performing a second (nested) PCR on the reaction product of said first PCR reaction,
- 30 (f) quantitatively and qualitatively measuring the presence of human ced-6 RNA by analysing the reaction products from the first and second PCR and
- (g) comparing the amount and type of reaction products formed in the first and second PCR
35 with that of the reaction products formed using phagocytes from control individuals.

Preferably, the PCR is performed with primers derived from the sequence of h1ced-6 or h2ced-6 as defined herein or derived from the vector used in the generation of cDNA. In particular said first PCR may be performed with primers having nucleotide sequences:

- 1) cgcaaggatcccatgaaccgtgcttttagcaggaag
- 2) gatctactaggtactggag

The second PCR may be performed with primers having nucleotide sequences:

- 1) cgcaaggatcccatgaaccgtgcttttagcaggaag
- 2) gcggatggtaccgtcgactgctgatacttgagttattctcag

The assay methods described herein, developed by the present inventors for determining whether compounds are enhancers or inhibitors of human CED-6 have been found to be more generally applicable for identifying compounds which influence phagocytosis of apoptotic cells by any mechanism, not necessarily related to human CED-6 or the signal transduction pathway of which it forms a component.

Liu et al. (Liu, Y. et. al, The American Association of Immunologists, 1:1999.) describe an assay for identifying compounds which influence phagocytosis of apoptotic cells, in which varying concentrations of the compound to be tested are added to the phagocytes which are subsequently seeded with apoptotic cells. To quantify phagocytosis of apoptotic cells, the authors used a microscopic quantification of phagocytosis in which the uptake of apoptotic cells was shown by electron microscopy and counted by light microscopy with a minimum of cells per slide being counted (Savill, J. S. Wyllie, A. H. Henson, J. E. Walport, M.

J.Henson, P. M.Haslett, C., J Clin Invest, 83:865-875, 1989.).

5 The presently known techniques for quantitating the phagocytosis of apoptotic cells do not readily lend themselves to high throughput screening of compounds for potential pharmacological activity. This is largely because the known assay techniques rely on microscopic counting of the proportion of phagocytes which have ingested apoptotic cells when exposed to the test compound. However, the present assays overcome this drawback because they can be performed in the multi-well assay format and provide detection systems which do not involve microscopy.

15 Thus, in accordance with a further aspect of the invention there is provided a method of identifying a compound which is an enhancer or inhibitor of phagocytosis of apoptotic cells which comprises:

20 a) exposing a mammalian professional or semi-professional phagocyte to an apoptotic mammalian cell which has been stably transfected with a reporter gene capable of generating a signal detectable without microscopy, in the presence or absence of the compound to be tested,

25 b) removing any apoptotic cells which are not engulfed by said phagocytes and

30 c) detecting any signal of the reporter gene from said phagocytes;

35 wherein any difference in signal in the presence of said compound compared to the signal in the absence of said compound is an indication that said compound is an inhibitor or an enhancer of phagocytosis of

apoptotic cells.

Usually, in the above method it is preferable to incubate the phagocytic cells with the test compound prior to addition of the apoptotic cells. A suitable incubation time might be about 15 to 30 minutes.

Suitable phagocytic cells for carrying out the method of the invention are mouse J774 cells or human THP-1 cells as described elsewhere herein. These cells are monocyte cell-lines but are cultured so as to differentiate them into macrophages prior to addition of apoptotic cells.

The apoptotic cells for use in the above method may be apoptotic neutrophils, apoptotic lymphocytes or apoptotic erythrocytes. The apoptotic cells may optionally be opsonised by exposure to serum. Preferred apoptotic cells are the adherent cell-lines L929 or PC12 and, in particular, the growth factor dependant mouse cell-line Ba/F3 described elsewhere herein.

The apoptotic cells are stably transfected with a reporter gene of the types and using the methods described above. One particular problem which can arise with the use of reporter genes is that expression of the gene in an apoptotic cell, which is effectively dying, can be much less than in a fully viable cell. If viable cells are present amongst the apoptotic particles added to the phagocytes and there is inadequate washing of the unphagocytosed particles the signal from the viable cells will mask any signal from the apoptotic phagocytosed cells. Although it is possible to ensure that adequate washing occurs the inventors have developed a particular embodiment where this problem is avoided.

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Specifically, this involves using a reporter gene expressing a protein, preferably an enzyme, with a low turnover in the cell such that the living cell and the apoptotic particles have approximately the same protein concentration or enzymatic activity. This overcomes the drawbacks described above. Several possible reporter proteins and substrates have been described in Handbook of fluorescent probes and research chemicals, ed by P. Haugland (Molecular probes, Eugene, OR, USA) which may be used. However, the inventors have found β -galactosidase (lacZ) to be particularly suitable. The enzyme has a relative slow turnover and it is shown that the cells and the apoptotic particles have relatively equal amounts of activity. Furthermore several substrates exist for β -galactosidase (see molecular probes, Eugene, OR, USA) from which the inventors have used FDG mentioned above. This makes it possible to develop a high throughput screen to select for compounds that alter the phagocytosis of apoptotic particles.

The phagocytes for use in the method of the invention may be wild-type cells or they may be transgenic or mutant cells. A mutant cell may have reduced or increased phagocytic activity compared to wild-type. A transgenic cell may be one stably transfected with a gene which when expressed influences the rate of phagocytic activity for apoptotic cells. For example, the mammalian cells may be transfected with h1ced-6 or h2ced-6 as described above, preferably using any of the vectors mentioned herein in the description or drawings.

In another embodiment in accordance with the invention the phagocytes may be transfected with a DNA encoding the cell surface antigen CD36. Expression of CD36 is required for phagocytosis of apoptotic cells by human

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macrophages that use either a phosphatidylserine receptor or the vitronectin receptor. (Fadok V.A. et al, J. Immunol 1998 Dec 1;161(11):6250-7.)

5 Transfection may be carried out by any of the methods described herein and preferably using a vector comprising a DNA sequence encoding CD36 as shown in Figure 31 or the entire vector of Figure 31.

10 The methods of the invention are all performed in a multi-well plate format and are therefore particularly suitable for mid-to-high throughput screening. In a preferred embodiment, the multi-well plates have 96 wells, but the invention is also applicable to multi-well plates with another number of wells, which
15 include but is not restricted to plates with 6, 12, 24, 384, 864, 1536 wells.

All of the methods of the invention require the detection of a signal which quantitates the
20 phagocytosis of apoptotic cells in the presence of the compound under test. It is an essential feature of the methods of this invention that this signal (also referred to as the read-out) is detected using a non-visual detection means. As used herein the term 'non-
25 visual detection means' refers to any means of detecting a signal which does not require visual inspection of the human eye including inspection through a microscope. The use of a non-visual detection system represents a major advantage over
30 previously known screening methods which require visual inspection of the cells by eye in order to detect uptake of apoptotic cells by phagocytes.

35 To allow for the non-visual detection of the apoptotic cells, in the high to mid-throughput screening in the phagocytosis assay, the reporter gene must be capable of generating a signal which is detectable by an

automatic plate reader, such as the victor2 (Wallac, Turku, Finland). An automatic plate reader which detects a fluorescent signal is most preferred.

- 5 By generating a signal which can be read by an automatic multiwell plate reader quantitative measurements can be made and this allows for the assessment of the effect of many compounds at once, as well as comparison of the effects between the
10 compounds.

15 It is further pointed out that the compounds to be tested may be any of the types of compounds described above and that where a particular compound results in a reduced signal or no signal for the phagocytes, the phagocytes will be tested for viability to rule out non-specific toxicity of the compound in question.

20 In the non-limiting examples which follow reference is made to the following Figures:

FIGURE 1 shows the construction of 2416bp consensus sequence which was obtained from EST, RACE and colony hybridization (see Example 1). The sequence was
25 compiled by using a al59394 as template and primers as indicated in multiple alignment. rcc stands for reverse complement. Both ced-6 and hced-6 are indicated above the multiple alignment; pGA101 was picked up by colony hybridization;

30 FIGURE 2 shows the consensus DNA sequence of h1ced-6 (2416 bp). Start and stop codons are in bold and underlined. Alternatively, spliced region is underlined;

35 FIGURE 3 shows the alternatively spliced DNA sequence of h2ced-6. Start and stop condon in bold and

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underlined;

FIGURE 4 shows the amino acid sequence of hCED-6.

Number of residues: 304, Molecular weight 34.4kDa.

5 again the alternatively spliced region is underlined;

FIGURE 5 shows the amino acid sequence of h2CED-6, the alternatively spliced version;

10 FIGURE 6 shows gel analysis of the nested PCR products generated as described in Example 3; the lanes are loaded as follows: (1) 100bp marker, (2) primary living neutrophils, (3) primary apoptotic neutrophils, (4) primary macrophages, (5) primary macrophages
15 interacted with apoptotic neutrophils, (6) J774, (7) COS-1, (8) THP-1;

FIGURE 7 shows the DNA sequence of the commercially available Clonotech vector pEGFP-N3 comprising the
20 reporter gene GFP as used in Examples 4 and 8;

FIGURE 8 shows a plasmid map of pEGFP-N3;

FIGURE 9 shows the DNA sequence of plasmid pGA3104, as
25 used in Examples 4 and 8, which comprises hlced-6 in the multicloning site of pEGFP-N3;

FIGURE 10 shows a plasmid map of pGA3104;

30 FIGURE 11 shows a DNA sequence of commercially available plasmid pCDNA3.1/His/LacZ used for stable transfection of Ba/F3 cells (see Example 4);

FIGURE 12 shows fluorescence intensity as a function of
35 transfected cell concentration when β -galactosidase is reacted with the fluorogenic substrate fluorescein di-b-D-galactopyranoside (FDG);

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FIGURE 13 shows the effect of (FDG) concentration on the read-out of the assay of Example 5;

5 FIGURE 14 shows the effect of incubation time on the read-out of the assay of Example 5;

FIGURE 15 shows the effect of serum concentration in medium of Ba/F3 cells on the assay of Example 5;

10 FIGURE 16 shows the location of the epitopes in h1CED-6 used for generating polyclonal antibodies;

FIGURE 17 shows the DNA sequence of the plasmid pGA1028 (pBAD/His A/-hced-6) used in Example 7;

15 FIGURE 18 shows a plasmid map of pGA 1028;

FIGURE 19 shows the DNA sequence of commercially available Promega plasmid pGL2 which is suitable for introduction of reporter gene luciferase into Ba/K3 cells;

20 FIGURES 20 to 25 show the results of the immunoblots carried out in Example 7. In all these figures the lanes are loaded as follows:

Lane 1: Prestained SDS - PAGE Standards Low Range (Bio Rad - Hercules, CA, USA),

30 Lane 2: pBAD/His A (Invitrogen, Leek, The Netherlands)

Lane 3: pGA1028 (pBAD/HisA/-h1CED-6)

35 FIGURE 20 shows gel stained with antibodies to the epitope EP 990044 as identified in Example 7 and control antibodies Anti-Xpress Ab (Invitrogen, Leek,

The Netherlands) and Mouse Ig, horseradish peroxidase-linked whole antibody (from sheep) (ECL Western blotting detection reagents and analysis system, Amersham Pharmacia Biotech, UK, England);

5

FIGURE 21 shows gel stained with antibodies to epitope 990044 and immune serum as described in Example 7;

10

FIGURE 22 shows gel stained with antibodies to epitope 990045 as identified in Example 7 and control antibodies as described for figure 20;

15

FIGURE 23 shows gel stained with antibodies to epitope 990045 and immune serum as described in Example 7;

FIGURE 24 shows gel stained with antibodies to epitope 990046 and with control antibodies as described for Figure 20;

20

FIGURE 25 shows gel stained with antibodies to epitope 990046 and with immune serum as described in Example 7;

25

FIGURE 26 shows the DNA sequence of the commercially available Clonotech vector pEGFP-C2 comprising the reporter gene GFP as used in Example 8;

FIGURE 27 shows a plasmid map of pEGFP-C2;

30

FIGURE 28 shows the DNA sequence of plasmid pGA3103 as used in Example 8 which comprises hIced-6 in the multicloning site of pEGFP-C2;

35

FIGURE 29 shows a plasmid map of pGA3103;

FIGURE 30 shows Western blot of cell lysates from COS-1 cells transfected with MOCK (negative control for

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transfection), pEGFP-N3, pGA3103 and pGA3104; control lysates for actual co-immunoprecipitation from Ba/F3 cells incubated with or without the first antibody; positive control lysates from EGF-stimulated A431 cell lysates for anti-phosphotyrosine antibody. Blot A was probed with a mouse monoclonal IgG2 which detects tyrosine-phosphorylated proteins in cell lysates; blot B was probed with polyclonal antibody which reacts with green fluorescent protein; and blot C was probed with rabbit antiserum to h1CED-6. MW of h1CED-6 is 34435.39; Mw of GFP is 26886.32; and Mw of the fusion protein GFP-CED-6 or CED-6-GFP is 62385.95;

FIGURE 31 shows the DNA sequence of plasmid pGA1058 which comprises a DNA sequence encoding the cell surface receptor CD36 inserted in the multicloning site of pEGFP-N3;

FIGURE 32 shows a plasmid map of pGA1058;

FIGURE 33 shows the percentage annexin and propidium iodide positive cells in a cell population of Ba/F3 cells as a function of time after withdrawal of IL-3;

FIGURE 34 shows the effect of temperature on FDG incubation, live and apoptotic Ba/F3 cells were added to macrophage cell-line J774. After the phagocytosis assay, FDG (10 μ M) was incubated for 1h at 4°C, 20°C and for 10 and 20 min at 37°C.

EXAMPLE 1

Extensive searches (tblastn) with the ced-6 sequence (Figure 2 Consensus DNA Sequence of hced-6) against the public domain databases (EST, Genbank, EMBL, Swissprot and PIR) revealed statistically significant homologies to some ESTS at the carboxyterminal region

of the protein (AA443368, AA431995, R33389, R53881).
One Est (T48513) showed homology to the
carboxyterminal of the PTB domain and the beginning of
the charged region. For 5' RACE analysis a Marathon-
ready cDNA colorectal adenocarcinoma, library was used
from Clontech. The position of the primers used for
RACE and sequencing is indicated in Figure 1. By
subsequent cloning and sequence analysis additional
sequence information was obtained. Using this
additional sequence information and subsequent rounds
of database searching (blastn) revealed additional
EST, which enabled us to construct a consensus of
approx. 2400 bp. This sequence was further extended
and verified by colony hybridization and sequencing
additional RACE products.

EXAMPLE 2

RNA Blots:

A human multiple tissue Northern (MTN-1, Clontech)
containing in each lane 2 mg of poly A + RNA from
eight different human tissues (heart, brain, placenta,
lung, liver, skeletal muscle, kidney, and pancreas)
and a MTN-II human multiple tissue Northern,
containing in each lane 2 mg of poly A + RNA from
spleen, thymus, prostate, testis, ovary, small
intestine, colon and peripheral leukocyte, were
hybridized according to the manufacturer's
instructions and washed out in 0.1 x SSC, 0.2% SDS at
55°C. Also from Clontech, a poly A + RNA blot from
human cancer cell lines (melanoma G361, lung carcinoma
A549, colorectal adenocarcinoma SW480, Burkitt's
lymphoma Raji, lymphoblastic Leukemia Molt-4, chronic
myelogenous leukemia K562, Hela S3 and promyelocytic
leukemia HL60) was tested.

Expression pattern of hCED-6 in normal human tissues.

and cancer cell lines by Northern blotting is shown in Table I below:

A) Human Multiple Tissue Northern (MTN) Blot B) Human Multiple Tissue Northern (MTN) Blot II C) Human Cancer Cell Line Multiple Tissue Northern (MTN™) Blot.

TABLE I

A)

	heart	brain	placenta	lung	liver	skeletal muscle	kidney	pancreas
Expression level	+		+++	+		++	+	+
length (kb)	3.6		3.6	3.6		3.9	3.6	3.6

B)

	spleen	thymus	prostate	testis	ovary	small intestine	colon (mucosal lining)	peripheral blood leukocyte
Expression level	+		+	++	+	+	+	
length (kb)	3.6		3.6	3.9	3.6	3.6	3.6	

C)

	promyelocytic leukemia HL-60	HeLa cell S3	chronic myelogenous leukemia K-562	lymphoblastic leukemia MOLT-4	Burkitt's lymphoma Raji	colorectal adenocarcinoma SW480	lung carcinoma A549	melanoma G361
Expression level		++	+++			+++	+++	+
length (kb)		3.6	3.6			3.6	3.6	3.6

EXAMPLE 3Detection of the CED-6 (h1CED-6) and its splice variant (h2CED-6) in phagocytic cell lines.

5

Cell line THP-1 (ATCC no: TIB-202), a human monocyte cell line that can be differentiated into a macrophage cell with PMA (Sigma-Aldrich, St-Louis, MO, USA), was cultivated under standard conditions in RPMI 160 medium containing 2mM L-glutamine, 1.5g/L sodium bicarbonate, 4.5 g/L glucose, 10mM HEPES, 1 mM Sodium pyruvate, 0.05mM β -mercaptoethanol. (all purchased from gibcoBRL, Life Technologies, Merelbeke, Belgium)

15

RNA has been isolated from this cell line using the RNeasy mini kit from qiagen (Westburg, Leusden, the Netherlands), according the instructions of the manufacture, or with minor modifications thereof.

20

Starting from this RNA, first strand cDNA was generated using the Ready-To-Go T-primed First-strand kit from Pharmacia Biotech (Piscataway, NJ, USA), according the instructions of the manufacture, or with minor modifications thereof.

25

The generated cDNA was used in a PCR protocol to generate DNA fragments using primers:

oGA131: 5'-CGCAAGGATCCCCATGAACCGTGCTTTTAGCAGGAAG-3'

445-10934-13R: 5'-GATCTACTAGGTACTGGAG-3'

30

PCR was performed with the TaKaRa ex Taq kit (Takara Shuzo CO., LTD, Shiga, Japan) according the instructions of the manufacture, or with minor modifications, Plasmid pGA1025, harboring the h1ced-6 gene was used as positive control.

35

- 30 -

In summary:

PCR on the first-strand cDNA isolated from the Ready-To-Go T-primed First-strand kit contained the entire
 5 cDNA as made, 0.4 μ l oGA131 (100pmol/ μ l), 0.4 μ l 445-10934-13R (100pmol/ μ l), 0.5 μ l exTaq 5U/ μ l, 65.7 μ l water.

PCR on the positive control contained, 10 μ l buffer
 10 exTaq 10x, 10 μ l dNTP mix exTaq 10x, 0.4 μ l oGA131 (100pmol/ μ l), 0.4 μ l 445-10934-13R (100pmol/ μ l), 2 μ l pGA1025, 76.2 μ l water.

PCR-program:

15 95°C 1' ┐
 45°C 30" ┘ 40x
 72°C 30" —
 72°C 7'
 4°C ∞

2 μ l of each PCR reaction and 1 μ l from the positive control PCR reaction was used to perform a nested PCR,
 20 with following primers:

oGA131: see above

oGA141: 5'-GCGGATGGTACCGTCGACTGCTGATACTTGAGTTATT
 CTCAG-3'

25 PCR was performed with the TaKaRa ex Taq kit (Takara Shuzo CO., LTD, Shiga, Japan) according the instructions of the manufacture, or with minor modifications.

The mastermix: 5 μ l buffer exTaq 10x, 5 μ l dNTP mix
 30 exTaq 10x, 0.2 μ l oGA131 (100pmol/ μ l), 0.2 μ l oGA141 (100pmol/ μ l), 0.5 μ l exTaq 5U/ μ l, 37.1 μ l water.

Program: 94°C 4'

35 94°C 1' ┐
 45°C 30" ┘ 35x
 72°C 30" —
 72°C 7'
 4°C ∞

10 μ l nested-PCR product was analyzed on gel using standard protocols (Molecular Cloning, a laboratory manual, Sambrook et al, 1989, CSHL press).

5 The above procedure was repeated for primary living neutrophils, primary apoptotic neutrophils, primary macrophages, primary macrophages interacted with apoptotic neutrophils, mouse monocyte cell-line J774 and COS-1 cells. The results are shown in Figure 6.
10 Remarkably, only in cell-line THP-1 could h1ced-6 and its splice variant h2ced-6 be detected (see lane 8 of Figure 6).

15 EXAMPLE 4

Stable cell lines of human CED-6

J774 murine monocyte tumour cell line (Morland and Kaplan, 1978, Exp. Cell Res. 115:53-61; Morland and Kaplan, 1978, Exp. Cell Res. 115:63-72 Accession No
20 ATCC TIB67 - also described as J774A.1) cultivated in DMEM, with glutamaxI, 10% myoclone serum (all from GIBCOBRL, Life Technologies, Merelbeke, Belgium), were transfected by electroporation, with the plasmids pEGFP-n3 (Clontech, Palo Alto, CA, USA) (Figures 7 and
25 8), mock transfection, pGA3104, h1CED-6/GFP fusion (Figures 9 and 10) Salmon Sperm DNA; negative control.

Electroporation was performed with Easyject Plus
30 electroporator system from Equibio Ltd (Immunosource, Halle-Zoersel, Belgium), using following protocol:
3 x 10⁶ cells were placed in 800 μ l cell culture medium, and 30 μ g DNA was added
The settings of the Easyject Plus electroporator were:
35 double pulse:

Voltage I = 750V, Capacitor I = 25 μ F, Resistance I = 99R, Interpulse delay= 0

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Voltage II 150 V, Capacitor II = 1500 μ F, Resistance II = 99R, Optipulse option
3200 μ l of electroporated cells per construct were seeded into a 175 cm² culture flask, and selected with
5 G418 antibiotic (400 μ g/ml) (Duchefa, Haarlem, The Netherlands) after 72h. Subclones of clones were obtained and checked for GFP expression.

EXAMPLE 5

10

Phagocytosis assay

Preparing the phagocytes

Monocyte cell line J774 stably transfected with pEGFPn3 or pGA3104, or pGA1058 were plated at a
15 density of 1×10^5 in a black 96-well plate for 36h at 37 °C and 5% CO₂ in advance of performing the phagocytosis assay.

Preparing the apoptotic cells

20 Growth factor dependent cell line Ba/F3 stably transfected with β -galactosidase as reporter gene is used as source of apoptotic cells. A suitable plasmid for transfecting Ba/F3 is pCDNA3.1/His/LacZ as shown in Figure 11. Ba/F3 cells which are IL-3 dependent
25 mouse clones (Palacios and Steinmetz, 1985, Cell 41:727-734; Palacios et al, 1984, Nature 309:126-131), were grown in DMEM with glutamaxI, 10% FCS, 1% antibiotics (all from GIBCOBRL, ibid.), and 10% supernatant from WEHI-3 culture.

30 WEHI-3 (ATCC no.: TIB-68) produces IL-3 when grown in culture medium: RPMI 1640 with glutamaxI, 10% FCS, 3.6 μ l β -mercaptoethanol per 1 litre.

Ba/F3 cells were split $\frac{1}{2}$ two days before the interaction assay (exponential growth phase) and Ba/F3
35 cells (5×10^6 /ml) were cultured without growth factor IL-3 for 20h in advance of performing the assay.

Apoptotic Ba/F3 cells were monitored by the

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annexin/propidium iodide labeling Kit from Boeringher-Mannheim (Brussels, Belgium). Ba/F3 cells are early apoptotic if 20% annexin positive and less than 5% propidium iodide negative. Ba/F3 cells cultured with growth factor IL-3 were used as a negative control. Results of the annexin/propidium iodide test are shown in Figure 33.

Adding the apoptotic cells to the phagocytes

100 μ l Ba/F3 cells (1×10^7 cells/ml) were added to wells containing stably transfected J774 and to the negative control wells and incubated at 37°C for periods ranging from 20 minutes to 5 hours after which the apoptotic cells were removed from all wells. Phagocytes were washed three times in PBS buffer (GIBCOBRL, ibid.), being careful not to dislodge any of the cells.

Read-out

Phagocytosis by the J774 cells of the apoptotic bodies was measured by detecting the β -galactosidase as expressed in the Ba/F3 cells. Detection was performed with a fluorogenic substrate, Fluorescein di-b-D-galactopyranoside (FDG) (Molecular probes, Eugene, OR, USA). 10 μ M FDG was added to the wells and incubated for 1h at room temperature in the dark. FDG is sequentially hydrolysed to FMG and fluorescein by the activity of the β -galactosidase, and the green fluorescein emission was measured in a standard plate reader using 480nm excitation, 520nm emission and the appropriate sensitivity settings.

For calibration purposes fluorescent read-out was determined for different Ba/F3 concentrations. The fluorescent read-out as a function of cell concentration is shown in Figure 12.

Further experiments were carried out varying the concentration of FDG, the incubation time of the

assay, the temperature of the assay and the concentration of serum in the Ba/F3 medium. The results are shown in Figures 13, 14, 15 and 34 respectively.

5

Compound Screening

10 The above described phagocytosis assay is used to screen compounds for their ability to influence the level of phagocytosis of apoptotic cells by professional phagocytes. The test compound or compounds can be added to the test wells approximately 30 minutes before addition of the Ba/F3 cells to the wells. Because of the multiwell format of the assay and automatic readout of fluorescence using standard equipment the assay is ideally suited to high throughput compound screening.

20 It will be understood that where any compound the presence of which results in no fluorescence or reduced fluorescence compared to phagocytic cells not exposed to the compound, the cells in that well will be tested for viability using commercially available reagents such as the Live/Dead Viability/Cytotoxicity Kit from Molecular Probes (Eugene, USA). This kit provides a two-colour fluorescence cell viability assay that is based on the simultaneous determination of live and dead cells with two probes, calcein AM and ethidium homodimer, that measure two recognised parameters of cell viability, intracellular esterase activity and plasma membrane integrity, respectively. This kit is suitable for use with fluorescence multiwell plate scanners. The presence of viable cells will confirm that the lack of fluorescence is due to the effect of the compound on phagocytic activity and not just non-specific toxicity.

35

- 35 -

Furthermore any compound identified as an inhibitor or an enhancer of phagocytosis of apoptotic cells by the assay described above will be further tested to confirm the effect is mediated through CED-6. In the case of a compound identified as an enhancer of phagocytosis of apoptotic cells this can be achieved by carrying out a phagocytosis assay exactly as described above with J774 cells which are not transfected with h1ced-6 or h2ced-6. If the compound is able to induce a phenotype in the J774 cells which is similar to the phenotype of those cells when transfected with hced-6 then it is an indication that the compound in question exerts its effect via CED-6 or via the CED-6 signal transduction pathway.

Similarly, if a compound identified in the above-described assay is an inhibitor of phagocytosis of apoptotic cells it can be confirmed whether its effect is via CED-6 or the CED-6 signal transduction pathway by examining the phenotype of the transfected J774 cells exposed to the compound. Reversion to a wild-type phenotype is an indication of action via CED-6 signal transduction pathway.

25 EXAMPLE 6

Polyclonal antibodies directed to human CED-6

Polyclonal antibodies were raised in rabbits against the following ced-6 epitopes:

30 EP990044 H2N - NRA FSR KKD KTC CONH2
EP990045 H2N - CFL GST EVE QPK GTE CONH2
EP990046 H2N - CTR NGT QPP PVP SRS T CONH2

Location of these epitopes in the ced-6 protein is shown in Figure 16.

35 The polyclonals were raised by Eurogentec Bel, Herstal, Belgium, using following protocol:
Day 0 : taking of pre-immune serum followed by the

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first immunisation

Day 14 : second immunisation

Day 28 : third immunisation

Day 38 : blood sampling (shipping 2ml)

5 Day 56 : fourth immunisation

Day 66 : blood sampling (shipping 2ml + 20ml)

Day 80 : complete bleeding.

EXAMPLE 7

10

Testing Antibodies by Western Blot

15

Transformation of plasmid pGA1028 (pBAD/His A-hCED-6) (see Figures 17 and 18) was done in TOP 10 competent cells. Furthermore pBAD/His was transformed in the same E. coli cells as a negative control. pBAS/HisA and E. coli TOP 10 were purchased from Invitrogen (Leek, The Netherlands).

20

25

30

The pBAD-vectors expression system was used, as it is known to be an efficient expression system. In the presence of arabinose, expression from pBAD is turned on while the absence of arabinose produces very low levels of transcription from pBAD. A pilot expression was carried out according the instructions of the manufacturer, in which the amount of arabinose was varied to determine the approximate amount of arabinose needed for maximum expression of your protein. The protocol according to the manufacturer (invitrogen, Leek, The Netherlands) was used. Expression was scaled up using the same protocol.

35

Purification of protein was performed from the E. coli cells transformed with pGA1028: 5 ml lysis buffer (10 ml TE 1x pH 8, 0.5 mg/ml lysozyme, 0.1mg/ml DNase, 100 µl 1M CaCl₂, 400 µl protease inhibitor 25x) was added to the pellet of a 50ml expression induced culture, and the pellet was resuspended in this lysis buffer.

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5 The suspension was placed for 30 mins on ice and
sonicated 3 times for 5" (high density), after which
the suspension was treated with 3 cycles of freeze-
defreeze (liquid nitrogen - 42°C), and placed for 30
min at 37°C. The suspension was centrifuged for 5' at
maximal speed. The pellet which contains the
insoluble fraction and also the hCED-6 fusion protein
was resuspended in 1 ml 2M urea and shaken for 5' at
1200 rpm. This suspension was centrifuged for 5' at
10 maximal speed, and the supernatant was used for gel
electrophoresis and Western blotting. 25 μ l supernatant
and 25 μ l premixed Laemmli Sample Buffer (Bio Rad-
Hercules, CA, USA) was mixed.

15 Proteins from the negative control were not purified.
E. coli transformed with pBAD/His were prepared by
pelletting 1 ml of induced E. coli culture, and
resuspending the pellet in 1 ml of premixed Laemmli
Sample Buffer (Bio Rad- Hercules, CA, USA). As such
20 this suspension can be used for PAGE Gel
electrophoresis.

Preparation of samples to load on a gel:
Both the samples were boiled for 5' and placed on ice
25 prior to loading. 25 μ l samples were loaded on a
Ready Gel, 50 μ l well TrisHCl, 4-15% (Bio Rad-
Hercules, CA, USA) and electrophoresis was performed
according to the manufacturer's instructions. The
proteins of the gel were transferred on nitrocellulose
30 membrane (Trans-blot Transfer medium, Bio Rad-
Hercules, CA, USA) with a MiniTransBlot
electrophoresis cell (Bio Rad- Hercules, CA, USA)
according to the instructions of the manufacturer (Bio
Rad- Hercules, CA, USA).

35 Western Blot was performed according to the providers
of the antibodies and the detection kit.

A first antibody, in the western blots, immune serum or pre-immune serum of the rabbits was used in a dilution of 1/2000 in PBST (1.44 g/L KH₂PO₄, 90 g/L NaCl, 7.75 g/L Na₂HPO₄·7H₂O, 0.1% Tween)

5 A second antibody: anti-rabbit, horseradish peroxidase-linked whole antibody (from donkey) diluted 1/4000 in PBST (0.1%) (ECL Western blotting detection reagents and analysis system, amersham pharmacia biotech, UK, England).

10 All incubations were performed according to the manufacturer, with the following modifications. The first antibody was incubated overnight in PBST, instead of TBST. Detection of the antibodies was done according to the manufacturer's instructions (ECL Western blotting detection reagents and analysis system, Amersham Pharmacia Biotech, UK, England).
15 Stripping and reprobing membranes after ECL detection kit, was done according to the manufacturer's instructions (ECL Western blotting detection reagents and analysis system, Amersham Pharmacia Biotech, UK, England).
20

Western Blot with control antibodies was done according the manufacturer's instructions of the anti-HisA antibodies (invitrogen, Leek, The Netherlands),
25 The antibody, designated Anti-Xpress antibody diluted 1/5000 in PBST (0.1%) (invitrogen, Leek, The Netherlands) was used as first antibody.

30 Second antibody: Mouse Ig, horseradish peroxidase-linked whole antibody (from sheep) diluted 1/4000 in PBST (0.1%) (ECL Western blotting detection reagents and analysis system, Amersham Pharmacia Biotech, UK, England).

The staining of the antibodies in both experiments
35 were performed according to the instructions of the manufacturer (ECL Western blotting detection reagents and analysis system, Amersham Pharmacia Biotech, UK,

England).

The results are shown in Figures 20 to 25.

5 EXAMPLE 8

Interaction of H-CED-6 With Phosphorylated Tyrosine Proteins

Co-immunoprecipitation

10 The antibodies raised against the three epitopes of h1CED-6 were used in western blotting to detect CED-6 interactions with phosphorylated tyrosine proteins and to identify CED-6 interacting proteins.

Transfection

15 Transfection of COS-1 cells was performed with plasmids pGA3103 (see Figures 26 to 29) and pGA3104 (see Figures 7 to 10) in a 175 cm² flask (1 x 10⁷ cells). As a negative control: MOCK and pEGFP-N3 were used. Full length human ced-6 (in frame with GFP, both N and C terminal fusions, internal control) were
20 investigated. COS-1 cells were transfected with lipofectamine Plus reagent (GIBCO-BRL). The protocol from Life Technologies was followed and the volumes that were used are shown in Table 2.

25 MOCK transfected cells are a negative control for transfection. In place of adding DNA, the solvent of the DNA only is added to the cells. Solvent of DNA is TE buffer, pH=8: 1M Tris (ICN) Ph=8 and 0.5 M EDTA (Merck-Belgolabo) pH=8 in H₂O.

30

TABLE 2

Lipofectamine transfection (Life Technologies) of COS-1 cells in a 175 cm² flask

Culture flask	Construct	Conc. DNA	DNA	DNA	Optimem	Plus reagent	Optimem	Lipo fecta mine	Optimem	Optimem
		µg/µl	µg	µl	µl	µl	µl	µl	µl	ml
175 cm ²	MOCK	-	-	12 (TE)	1125	60	1125	90	2250	15
175 cm ²	pEGFP-N3	1	12	12	1125	60	1125	90	2250	15
175 cm ²	PGA3103	1	12	12	1125	60	1125	90	2250	15
35 175 cm ²	PGA3104	1	12	12	1125	60	1125	90	2250	15

- 40 -

As a positive control for phosphorylation, the β -chain of the IL-3 receptor of Ba/F3 cells which is phosphorylated was used.

- 5 COS-1 cell lysates were prepared using DIGITONIN (as gently as possible, not to disturb the interaction) and phosphatase inhibitors added (protease inhibitors, preferably cocktailpils or pefablock) to the lysis buffer.

10

- Low stringency DIGITONIN based buffer:

Buffer with DIGITONIN in 10 ml bidl

15

- 1 % digitonin (Serva 19551, MW 1229.3) SERVA
2% stock= 250 mg in 12.5 ml
- 10 mM triethanolamine pH 7.8 (Sigma-Aldrich;
Bornem, Belgium) 10X stock 100 mM= 185,7 mg in
10ml pH 7.8 (5ml in 50ml)
- 0.15 M NaCl (MW 58.44) 87.66 mg per 10ml
- 2 mM Na_3VO_4 (Sigma-Alrich, Bornem , Belgium)
3.687 mg per 10 ml
- 2 mM EDTA (Titriplex III; MW 372.24)
(Darmstadt, Germany)
7.444 mg per 10 ml
- 200 U/ml aprotinin - Trazilol (Sigma-Aldrich,
Bornem, Belgium)
1 mg = 11 TIU = 9900 KU
200X stock: 10 mg in 2 ml PBS = 49500 KU (50 μ l
in 10ml)
- 1 mM Pefabloc (Merck , Darmstadt, Germany) 2.4
mg per 10 ml

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25

30

Lysis of cells

- Transfected cells were washed 2 x in PBS Dulbecco's (GIBCOBRL) in falcon
- 35 • Cells were scraped and pellet resuspended in 300 μ l lysis buffer.

- All manipulations were carried out at 4°C.
- The preparation was centrifuged at 4000 rpm and the supernatant transferred to a new tube.

Preclearance

- 5 • Protein G sepharose CL-4B beads (Amersham Pharmacia, Roosendaal, the Netherlands) were supplied freeze dried in the presence of additives. These additives were washed away at neutral pH and ethanol replaced with lysis buffer.
- 10 • 50% v/v Protein G sepharose suspension:
1 ml 50/50 v/v Protein G sepharose was pipetted and centrifuged at high speed for 5 sec. It was then aspirated and resuspended in equal volume of lysis buffer. Washing was repeated three times.
- 15 • To 300 μ l of lysate was added 50 μ l of protein G sepharose CL-4B beads (Amersham Pharmacia, ibid.) and this was reacted for 1 hour.
- It was then centrifuged 10 sec at 14 000 rpm and 4°C and the supernatant transferred to a new tube.

20

Co-immunoprecipitation

- COS-1 lysates: 5 μ l anti-green fluorescent protein (GFP) polyclonal antibody rabbit (Immunosource, Halle-Zoersel, Belgium) was added.
- 25 • Lyophilized form was dissolved in 100 μ l distilled water then frozen at -20°C
- Ba/F3 lysate number 5: 5 μ l of rat antibody to β -chain of IL-3 receptor (Van der Heyden J., Devos R., Plaetinck G., Fache I., Fiers W., Tavernier J. 1991. Characterization of the murine IL-5 receptor complex with the use of a panel of monoclonal antibodies. Relationship to the murine IL-3 receptor. J Immunol. 147:3413-3418) was added.

30

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- Ba/F3 lysate number 6: no antibody was added.
- Samples were incubated between 4 h and 24 h (overnight) at 4 °C, rotating
- 5 • 50 µl protein A beads were added and incubated for 1 hour at 4°C.
- Samples were centrifuged for 3 min at 3000 rpm (4°C)
- Beads were resuspended in 800 µl lysis buffer, inverted several times or rotated for a few minutes and centrifuged at 3000 rpm for 3 minutes (4°C).
- 10 This was repeated three times and on the last occasion the wash buffer was removed with a capillary tip.
- Beads were suspended in 20 µl SDS loading buffer (with -mercapto)
- 15 • Lysate number 7= EGF-stimulated A431 Cell lysate (positive control for anti-phosphotyrosine) (Upstate Biotechnology, cat. No. 12-302)
- 2.5µl of β-mercaptoethanol was added to 100µl of lysate and samples boiled.
- 20 • Samples were centrifuged for 3 min at 3000 rpm (4 °C) and SN was loaded on SDS PAGE using prestained SDS-Page standards low range from BioRad (cat.no 161-0305)

25

Western-blotting

- Cell lysates were transferred to nitrocellulose with:
- 30 • Transfer buffer= 48 mM Tris, 39 mM glycine, 20% methanol, pH 9.2 (5.82 g Tris, 2.93 g glycine in H₂O, 200 ml methanol, to 1 L H₂O)
- Blocking buffer: 1x PBS, 0.1% Tween, 5% milk powder, incubate blot overnight
- 35 • Gel was probed with anti-phosphotyrosine (cat. 05-321, Upstate Biotechnology): 1 µg/ml for 3h and

- 43 -

washed twice with PBS, 0.1% Tween for 5 min

- Proteins were visualized using 1:4000 goat anti-mouse horseradish peroxidase (cat.no RPN 2108, Amersham pharmacia biotech) as second Ab for 1h at RT.

- Blots were washed twice with PBS, 0.1% Tween for 5 min, twice with blocking buffer for 5 min and twice with H₂O (5min)

10 ECL Western blotting analysis system

ECL Western blotting detection reagents from Amersham Pharmacia Biotech (cat.no RPN 2108) were used.

15 Stripping and reprobing blot after ECL detection kit

The membranes were stripped of bound antibodies and reprobed. Membranes were stored wet in saran wrap at 4°C after each immunodetection.

- 20 • The membrane was submerged in stripping buffer (100 mM β -mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7) and incubated at 50 °C for 30 min with occasional agitation.
- The membrane was washed for 2 x 10 min in PBS, 0.1% Tween at room temperature using large volumes of wash buffer.
- 25 • The membrane was blocked by immersing in blocking buffer for 1h at RT.
- Immunodetection was performed with anti-green
- 30 fluorescent GFP and repeated with anti-human CED-6

Results

35 Western blots of all cell lysates probed either with anti-phosphotyrosine, anti-green fluorescent protein (GFP) or with rabbit sera against CED-6 are shown in

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Figure 30, blots (A), (B) and (C). One band between 49 and 74K stained with anti-phosphotyrosine is present in the COS-1 cell lysates transfected with fusion proteins of GFP and CED-6 and is not present in the control COS-1 cell lysates. By probing the western blot with anti-GFP and anti-CED-6 the same band between 49 and 74K was stained.

Conclusion

Fusion proteins CED-6-GFP and GFP-CED-6 are both tyrosine phosphorylated. Their molecular weight is 62385.95K which represents the band between 49 and 74K that is stained positive for anti-phosphotyrosine, anti-green fluorescent protein (GFP) and anti-CED-6.

EXAMPLE 9

Stable cell lines transfected with human cell surface receptor CD36.

J774 murine monocyte tumour cell line was transfected by electroporation with plasmid pGA1058 shown in Figure 31 and 32. The methods used were as described in example 4 for human ced-6.

The transfected cell-line was used as a positive control in carrying out phagocytosis assays using the protocol of Example 5.

EXAMPLE 10

Generation of apoptotic particles starting from PC12.

The PC-12 cell-line(ATCC number: CRL-1721) (Mesner P.W., Winters T.R., Green S.H., (1992) J. Cell Biol.119:1669-1680, tends to grow in small clusters.

By addition of nerve growth factor-beta (50ng/ml final conc., Sigma), PC-12 cells differentiate into neuronal cells. By withdrawal of nerve growth factor after 5 days of treatment, programmed cell death in neuronal rat PC12 cells is induced.

The cells are cultured in RPMI 1640 (Life Technologies) with 2mM L-glutamine adjusted to contain 1.5g/L sodium bicarbonate, 4.5g/L glucose, 10mM HEPES and 1mM sodium pyruvate, 10% horse serum, 5% fetal bovine serum.

These cells can be tested for apoptotic character using the annexin/PI kit described above.

SEQUENCE LISTING

The nucleotide and amino acid sequences shown in the
Figures herein are designated the following SEQ ID
Nos.

5	SEQ ID NO: 1	Figure 1
	SEQ ID NO: 2	Figure 2
	SEQ ID NO: 3	Figure 3
10	SEQ ID NO: 4	Figure 4
	SEQ ID NO: 5	Figure 5
	SEQ ID NO: 6	Figure 7
	SEQ ID NO: 7	Figure 9
	SEQ ID NO: 8	Figure 11
15	SEQ ID NO: 9	Figure 17
	SEQ ID NO: 10	Figure 19
	SEQ ID NO: 11	Figure 26
	SEQ ID NO: 12	Figure 28
	SEQ ID NO: 13	Figure 31

20

CLAIMS:

1. An expression vector comprising a sequence of deoxynucleotides encoding a human CED-6 protein comprising the amino acid sequence of Figure 4 or Figure 5 or an amino acid sequence which differs from the amino acid sequence of Figure 4 or Figure 5 only in amino acid changes which are conservative of function.
2. An expression vector as claimed in claim 1 comprising the sequence of deoxynucleotides shown from the transcription start codon to the transcription stop codon in Figure 2 or Figure 3.
3. An expression vector as claimed in claim 1 or claim 2 which comprises a sequence of deoxynucleotides encoding a reporter gene positioned in said vector such that expression of said human CED-6 protein or functionally conserved variant thereof results in expression of a reporter protein from said reporter gene.
4. An expression vector as claimed in claim 3 wherein said reporter gene is positioned 5' to the sequence of deoxynucleotides encoding said human CED-6 protein or functionally conserved variant thereof.
5. An expression vector as claimed in claim 3 wherein said reporter gene is positioned 3' to the sequence of deoxynucleotides encoding said human CED-6 protein or a functionally conserved variant thereof.
6. An expression vector as claimed in any of claims 3 to 5 wherein said reporter gene encodes green fluorescent protein (GFP).

7. An expression vector as claimed in claim 4 which is pEGFP-C2 with a sequence of deoxynucleotides encoding a human CED-6 protein which comprises the sequence of amino acids as shown in Figure 4 or
5 Figure 5 or a functionally conserved variant thereof inserted in the multicloning site.

8. An expression vector as claimed in claim 5 which is pEGFP-N3 with a sequence of deoxynucleotides
10 encoding a human CED-6 homologue which comprises the sequence of amino acids as shown in Figure 4 or Figure 5 or a functionally conserved variant thereof inserted in the multicloning site.

15 9. An expression vector as claimed in claim 4 or 7 wherein said vector comprises the nucleotide sequence of Figure 28 (pGA3103).

10 10. An expression vector as claimed in claim 5 or 8 wherein said vector comprises the nucleotide sequence of Figure 9 (pGA3104).

25 11. An expression vector as claimed in claim 1 or claim 2 wherein the human CED-6 protein or functionally conserved variant thereof expressed from said vector includes an epitope tag at the amino and/or the carboxy terminus thereof.

30 12. An expression vector as claimed in claim 11 wherein said epitope tag is His A.

35 13. An expression vector as claimed in claim 11 which is pBAD/HisA with a sequence of deoxynucleotides encoding a human CED-6 protein comprising the sequence of amino acids as shown in Figure 4 or Figure 5, or a functionally conserved variant thereof, inserted therein.

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14. An expression vector as claimed in claim 12 which has the sequence of deoxynucleotides shown in Figure 17 (pGA 1028).

5 15. A mammalian cell-line transfected with an expression vector as claimed in any one of claims 1 to 13.

10 16. A mammalian cell-line as claimed in claim 15 wherein said cell is selected from a fibroblast cell-line or an epithelial cell line.

15 17. A mammalian cell-line as claimed in claim 16 wherein said cell-line is selected from COS1, BHK21, L929, CV1, SWISS 3T3, HT144, IMR32, HEPG2, MDCK, MCF7, 293, Hela, A549, SW48 or G361.

20 18. A mammalian cell-line as claimed in claim 15 wherein said cell-line is a primary cell-line.

25 19. A mammalian cell-line as claimed in claim 18 wherein said cell-line is selected from human dermal FIBs, dermal keratinocytes, leucocytes, monocytes, lymphocytes, dendritic cells or macrophages.

30 20. A mammalian cell-line as claimed in claim 19 which is mouse macrophage cell-line J774 or human monocyte cell-line THP-1.

35 21. A method for determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells which method comprises exposing transfected mammalian cells as claimed in any one of claims 15 to 20 to apoptotic particles and measuring the rate of phagocytic uptake of said particles by

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said transfected cells in the presence and absence of said compound.

5 22. A method as claimed in claim 21 wherein said transfected cells are exposed to said compound prior to addition of said apoptotic particles.

10 23. A method as claimed in claim 21 or 22 wherein said apoptotic particles are selected from the group consisting of apoptotic neutrophils, apoptotic lymphocytes and apoptotic erythrocytes which optionally have been opsonised.

15 24. A method as claimed in any of claims 21 to 23 wherein said apoptotic particles comprise adherent cell-line PC12.

20 25. A method as claimed in any of claims 21 to 23 wherein said apoptotic particles comprise the growth factor dependent mouse cell-line Ba/F3.

25 26. A method as claimed in claim 25 wherein said Ba/F3 cells are rendered apoptotic by culturing in the absence of growth factor IL-3.

30 27. A method as claimed in any one of claims 23 to 26 wherein the cells comprising said apoptotic particles are stably transfected with a reporter gene.

35 28. A method as claimed in claim 27 wherein said reporter gene is selected from a gene encoding β -galactosidase, a gene encoding a fluorescent protein or a gene encoding a protein capable of generating luminescence.

29. A method as claimed in claim 28 wherein

said protein capable of generating luminescence is luciferase.

5 30. A method as claimed in claim 28 wherein said fluorescent protein is green fluorescent protein.

10 31. A method as claimed in claim 26 wherein said apoptotic particles comprises Ba/F3 cells stably transfected with β -galactosidase or luciferase.

15 32. A method as claimed in claim 31 wherein the level of phagocytosis is detected by adding a substrate which is converted by said β -galactosidase to a fluorescent compound.

20 33. A method as claimed in any one of claims 21 to 32 wherein if no phagocytosis or a reduced amount of phagocytosis is observed on exposure to the test compound, said mammalian transfected cells are examined for viability.

25 34. A method as claimed in claim 33 wherein if viable the phenotype of said mammalian transfected cells is compared with the phenotype of untransfected mammalian cells of the same cell-line.

30 35. A method as claimed in any of claims 21 to 32 wherein if an increased amount of phagocytosis is observed in the presence of the test compound, the method comprises the further steps of exposing said compound to an untransfected mammalian cell of the same cell-line and observing whether the compound induces a phenotype which is substantially the same
35 as the phenotype exhibited by said transfected mammalian cell.

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36. A compound identified by the method of any of claims 21 to 35 as an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells.

37. A method for determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cell which method comprises the steps of:

(1) micro-injecting into a mammalian cell a human CED-6 protein comprising the sequence of amino acids shown in Figure 4 or Figure 5 or a sequence of amino acids differing from that shown in Figure 4 or Figure 5 only in amino acid changes conservative of function.

(2) exposing the mammalian cell produced in step (1) to apoptotic particles and measuring the rate of phagocytic uptake of said particles by said cell in the presence and absence of said compound.

38. A method as claimed in claim 37 wherein said micro-injected mammalian cells are exposed to said compound prior to addition of said apoptotic particles.

39. A method as claimed in claim 38 wherein said apoptotic particles are selected from apoptotic neutrophils, apoptotic lymphocytes and apoptotic erythrocytes which optionally have been opsonized.

40. A method as claimed in any of claims 37 or 38 wherein said apoptotic particles comprise adherent cell-line PC12.

41. A method as claimed in any of claims 37 to 39 wherein said apoptotic particles comprise the growth factor dependent mouse cell-line Ba/F3.

5 42. A method as claimed in claim 41 wherein said Ba/F3 cells are rendered apoptotic by culturing in the absence of growth factor IL-3.

10 43. A method as claimed in any one of claims 39 to 42 wherein the cells comprising said apoptotic particles are stably transfected with a reporter gene.

15 44. A method as claimed in claim 27 wherein said reporter gene is selected from a gene encoding β -galactosidase, a gene encoding a fluorescent protein and a gene encoding a protein capable of generating luminescence.

20 45. A method as claimed in claim 44 wherein said protein capable of generating luminescence is luciferase.

25 46. A method as claimed in claim 44 wherein said fluorescent protein is green fluorescent protein.

30 47. A method as claimed in claim 42 wherein said apoptotic particles comprise Ba/F3 cells stably transfected with β -galactosidase.

35 48. A method as claimed in claim 47 wherein the level of phagocytosis is detected by adding a substrate which is converted by said β -galactosidase to a fluorescent compound.

49. A method as claimed in any of claims 37 to

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48 wherein the mammalian cell is a fibroblast cell or an epithelial cell.

5 50. A method as claimed in claim 49 wherein the mammalian cell is selected from COS1, BHK21, L929, CV1, SWISS 3T3, HT144, IMR32, HEPG2, MDCK, MCF7, 293, Hela, A549, SW48 or G361.

10 51. A method as claimed in any of claims 37 to 48 wherein said mammalian cell is a primary cell.

15 52. A method as claimed in claim 51 wherein said mammalian cell is selected from human dermal FIBs, dermal keratinocytes, leucocytes, monocytes or macrophages.

20 53. A method as claimed in claim 52 wherein said mammalian cell is a mouse macrophage cell J774 or a human monocyte cell THP-1.

25 54. A method as claimed in any one of claims 37 to 53 wherein if no phagocytosis or a reduced amount of phagocytosis is observed on exposure to the test compound, said mammalian transfected cells are examined for viability.

30 55. A method as claimed in claim 54 wherein, if viable, the phenotype of said mammalian transfected cells is compared with the phenotype of untransfected mammalian cells of the same cell-line.

35 56. A method as claimed in any of claims 21 to 32 wherein if an increased amount of phagocytosis is observed in the presence of the test compound, the method comprises the further steps of exposing said compound to an untransfected mammalian cell of the same cell-line and observing whether the compound

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induces a phenotype which is substantially the same as the phenotype exhibited by said transfected mammalian cell.

5 57. A compound identified by the method of any of claims 37 to 56 as an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells.

10 58. A method for determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells which method comprises the steps of:

15 (1) micro-injecting or transfecting into a mammalian cell a vector expressing RNA antisense to all or a portion of the sequence of nucleotides shown in Figure 2 or Figure 3;

20 (2) exposing the mammalian cell produced in step (1) to apoptotic particles and measuring the rate of phagocytic uptake of said particles by said cell in the presence or absence of said compound.

25

59. A method as claimed in claim 58 wherein said antisense RNA comprises a sequence of nucleotides which is capable of hybridizing to a sequence of nucleotides as shown in Figure 2 or Figure 3 under conditions of stringency which are higher than 2xSSC; 0.1% SDS; 25°C to 50°C.

30

60. A method as claimed in claim 58 or claim 59 comprising the features of any one of claims 38 to 56.

35

61. A compound identified by the method claims 58 to 60 as an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells.

5 62. A peptide which comprises a fragment of a human CED-6 homologue having an amino sequence as shown in Figure 4 wherein said fragment includes the sequence of amino acids NRAFSRKKDKTC, FLGSTEVEQPKGTE
10 or TRNGTQPPPVPSRST.

63. A peptide as claimed in claim 62 consisting of the sequence of amino acids NRAFSRKKDKTC, FLGSTEVEQPKGTE or TRNGTQPPPVPSRST.

15 64. An antibody preparation comprising antibodies directed to one or more of the following epitopes of human CED-6 homologue as shown in Figure 4: NRAFSRKKDKTC, FLGSTEVEQPKGTE or TRNGTQPPPVPSRST

20 65. An antibody preparation comprising antibodies directed to the human CED-6 homologue epitope NRAFSRKKDKTC.

25 66. An antibody preparation comprising antibodies directed to the human CED-6 homologue epitope FLGSTEVEQPKGTE.

30 67. An antibody preparation comprising antibodies directed to the human CED-6 homologue epitope TRNGTQPPPVPSRST.

35 68. An antibody preparation as claimed in any one of claims 63 to 66 wherein said antibodies are polyclonal antibodies.

69. A method for diagnosing a disease

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associated with the over or under expression of human CED-6 protein in phagocytic cells in an individual which comprises:

- 5 (a) obtaining a sample of phagocytes from said individual;
 - (b) exposing said phagocytes to an antibody preparation as claimed in any of claims 64
10 to 68;
 - (c) quantitatively measuring the presence of any immune complexes formed between said antibodies and said CED-6 protein; and
15 (d) comparing the amount of immune complex formed with that formed using phagocytes from a control individual.
- 20 70. A method for determining whether a compound is an inhibitor or an enhancer of a signal transduction pathway which promotes phagocytosis of apoptotic cells which method comprises:
- 25 (a) exposing a mammalian cell transfected with an expression vector as claimed in any one of claims 1 to 14 to the compound to be tested;
 - 30 (b) exposing said mammalian cell to an antibody preparation as claimed in any of claims 64 to 68;
 - 35 (c) quantitatively measuring the presence of any immune complex formed between said antibodies and protein expressed by said cells; and

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5 (d) comparing the level of immune complex
detected with the amount of immune complex
detected in a mammalian cell transfected as
described in step (a) which has not been
exposed to said compound.

10 71. A method as claimed in claim 70 wherein
said mammalian cell is selected from COS1,
BHK21, L929, CU1 SWISS 3T3, HT144, IMR32,
HEPG2, MDCK, MCF7, 293, Hela, A549, SW48 or
G361.

15 72. A method as claimed in claim 71 wherein
said mammalian cell is a COS1 cell.

73. A method as claimed in claim 70 wherein the
mammalian cell is a human dermal FIB, dermal
keratinocyte, leucocyte, monocyte or macrophage.

20 74. A method as claimed in claim 73 wherein
said cell is mouse monocyte cell J774 or human
monocyte cell THP-1.

25 75. A fusion protein which comprises:
(1) a sequence of amino acids as shown in
Figure 4 or Figure 5 or a sequence of amino
acids which differs from the sequence shown
in Figure 4 or Figure 5 only in amino acid
changes conservative of function; and
30 (2) a protein which is the expression product
of a reporter gene.

35 76. A fusion protein as claimed in claim 75
which is obtained by expression of the GFP and hlced-
6 encoding sequences shown in Figures 9 or 28.

77. A fusion protein which comprises:

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(1) a sequence of amino acids as shown in Figure 4 or Figure 5 or a sequence of amino acids which differs from the sequence shown in Figure 4 or Figure 5 only in amino acid changes conservative of function, and

(2) an epitope tag.

78. A fusion protein as claimed in claim 77 which is obtainable by expression of the HisA and hIced-6 encoding sequences shown in Figure 17.

79. A method of identifying a compound which is an enhancer or an inhibitor of phagocytosis of apoptotic cells which comprises:

- a) exposing a mammalian professional or semi-professional phagocyte to an apoptotic mammalian cell which has been stably transfected with a reporter gene capable of generating a signal detectable without microscopy, in the presence and absence of the compound to be tested,
- b) removing any apoptotic cells which are not engulfed by said phagocytes and
- c) detecting any signal of the reporter gene from said phagocytes;

wherein any difference in signal in the presence of said compound compared to the signal in the absence of said compound is an indication that said compound is an inhibitor or an enhancer of phagocytosis of apoptotic cells.

80. A method as claimed in claim 79 wherein

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said phagocyte is mouse macrophage cell-line J774 or human monocyte cell-line THP-1.

5 81. A method as claimed in claim 80 wherein the monocyte cell-line is cultured under conditions to differentiate it into macrophages prior to exposure to said apoptotic particles.

10 82. A method as claimed in any of claims 79 to 81 wherein said phagocyte is a transgenic cell.

15 83. A method as claimed in claim 82 wherein said phagocyte has been transfected with an expression vector as claimed in any of claims 1 to 14.

20 84. A method as claimed in claim 82 wherein said phagocyte has been transfected with an expression vector encoding the cell surface receptor CD36.

25 85. A method as claimed in claim 84 wherein said phagocyte has been transfected with a vector as shown in Figure 31.

86. A method as claimed in any of claims 79 to 85 wherein said apoptotic cells comprise the adherent cell-line PC12.

30 87. A method as claimed in any one of claims 79 to 85 wherein said apoptotic cells comprise the growth factor dependent mouse cell-line Ba/F3.

35 88. A method as claimed in claim 26 wherein said Ba/F3 cells are rendered apoptotic by culturing in the absence of the growth factor IL-3.

89. A method as claimed in claim 88 wherein said cells are considered apoptotic if about 20% annexin positive and less than about 5% propidium iodide negative.

5 90. A method as claimed in any of claims 79 to 89 wherein said reporter gene is selected from a gene encoding β -galactosidase, a gene encoding a fluorescent protein or a gene encoding a protein
10 capable of generating luminescence.

91. A method as claimed in claim 90 wherein said protein capable of generating luminescence is luciferase.

15 92. A method as claimed in claim 91 wherein said apoptotic cell has been stably transfected with a plasmid exhibiting the expression characteristics of PGL2control shown on Figure 19.

20 93. A method as claimed in claim 92 wherein said apoptotic cell has been stably transfected with a plasmid comprising the sequence of deoxynucleotides shown in Figure 19.

25 94. A method as claimed in any one of claims 79 to 89 wherein fluorescent protein is green fluorescent protein (GFP).

30 95. A method as claimed in claim 94 wherein said apoptotic cell has been stably transfected with a plasmid exhibiting the expression characteristics or a plasmid as shown in Figure 10 or Figure 29.

35 96. A method as claimed in claim 95 wherein said apoptotic cell has been transfected with a plasmid comprising the sequence of nucleotides shown

in Figure 9 of Figure 28.

5 97. A method as claimed in any of claims 79 to 89 wherein said apoptotic cells have been stably transfected with a plasmid expressing b-galactosidase.

10 98. A method as claimed in claim 97 wherein said plasmid has the expression characteristics of the plasmid shown in Figure 11.

15 99. A method as claimed in claim 98 wherein said plasmid comprises the sequence of deoxynucleotides shown in Figure 11.

20 100. A method as claimed in any of claims 79 to 89 wherein said apoptotic particles comprise cell-line Ba/F3 stably transfected with β -galactosidase.

25 101. A method as claimed in claim 100 wherein the level of phagocytosis is detected by adding a substrate which is converted by said b-galactosidase to a fluorescent compound.

30 102. A method as claimed in any one of claims 79 to 101 wherein if no phagocytosis or a reduced amount of phagocytosis is observed on exposure to the test compound, said phagocytes are tested for viability.

35 103. A method as claimed in any of claims 79 to 102 wherein said phagocytes are cultured in multiwell plates the apoptotic cells and the test compounds being added to the individual wells thereof.

104. A method as claimed in any preceding claim

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wherein the signal from said reporter gene is detected by an automatic plate reader.

5 105. A method as claimed in claim 101 wherein the signal from the reporter gene is detected by an automatic plate reader capable of detecting a fluorescent signal.

10 106. A compound identified as an inhibitor or enhancer of phagocytosis of apoptotic cells by the method of any claims 79 to 105.

15 107. A method as claimed in any of claims 22 to 35, 39 to 56, and 58 to 60 wherein phagocytic uptake is measured by non-microscopic means.

20 108. A method as claimed in claim 107 wherein said non-microscopic means is a multi-well plate reader.

25 109. A method as claimed in claim 108 wherein said multi-well plate reader measures luminescence, fluorescence or performs spectrophotometric detection.

110. A method as claimed in any of claims 79 to 105 having the features of claims 108 and 109.

30 111. A method for diagnosing a disease associated with the over- or under-expression of human CED-6 in phagocytic cells in an individual, which method comprises:

- (a) obtaining a sample of phagocytes from said individual,
- 35 (b) isolating RNA from said phagocytes,
- (c) preparing cDNA from said RNA,
- (d) performing a first PCR reaction on said

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cDNA,

- 5 (e) performing a second (nested) PCR on the reaction product of said first PCR reaction,
- (f) quantitatively and qualitatively measuring the presence of CED-6 RNA by analysing the reaction products from the first and second PCR,
- 10 (g) comparing the amount and type of reaction products formed in the first and second PCR with that of the reaction products formed using phagocytes from control individuals.

15 112. A method as claimed in claim 111 wherein said PCR is performed with primers derived from the sequence of human CED-6, or derived from the vector used in the generation of cDNA.

20 113. A method as claimed in claim 111 or 112 wherein said first PCR is performed with primers having nucleotide sequences:

- 1) cgcaaggatcccatgaaccgtgcttttagcaggaag
- 2) gatctactaggtactggag

25 114. A method as claimed in any of claims 111, 112 or 113 wherein said second PCR is performed with primers having nucleotide sequences:

- 1) cgcaaggatcccatgaaccgtgcttttagcaggaag
- 2) gcggatggtaccgtcgactgctgatacttgagttattctcag

30

FIG. 1

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	1				50
consensus	GGTGATGAGC	CCTTGGGTTC	TCGCTCCGAC	TGCTAAATTC	GCTTGGCCGG
Seq	GGTGATGAGC	CCTTGGGTTC	TCGCTCCGAC	TGCTAAATTC	GCTTGGCCGG
thc117484	..TGATGAGC	CCTTGGGTTC	TCGCTCCGAC	TGCTAAATTC	GCTTGGCCGG
r65982GCTAAATTC	GCTTGGCCGG
aa159394	GGTGATGAGC	CCTTGGGTTC	TCGCTCCGAC	TGCTAAATTC	GCTTGGCCGG
aa369714	..TGATGAGC	CCTTGGGTTC	TCGCTCCGAC	TGCTAAATTC	GCTTGGCCGG
	51				100
consensus	GTCCACCTTC	TCGTGGCCTC	ACTCGCCACA	CGGATCAGAA	TCCGGAGCAG
Seq	GTCCACCTTC	TCGTGGCCTC	ACTCGCCACA	CGGATCAGAA	TCCGGAGCAG
thc117484	GTCCACCTTC	TCGTGGCCTC	ACTCGCCACA	CGGATCAGAA	TCCGGAGCAG
r65982	GTCCACCTTC	TCGTGGCCTC	ACTCGCCACA	CGGATCAGAA	TCCGGAGCAG
aa159394	GTCCACCTTC	TCGTGGCCTC	ACTCGCCACA	CGGATCAGAA	TCCGGAGCAG
aa369714	GTCCACCTTC	TCGTGGCCTC	ACTCGCCACA	CGGATCAGAA	TCCGGAGCAG
	101				150
consensus	GCAGTTCTCT	CTATTCTGAG	GCTCCTGCGG	C TGCC GCG	CTGACTTCCC
Seq	GCAGTTCTCT	CTATTCTGAG	GCTCCTGCGG	C TGCC GCG	CTGACTTCCC
thc117484	GCAGTTCTCT	CTATTCTGAG	GCTCCTGCGG	C.TGCCGCGC.	TGACTTCCC.
r65982	GCAGTTCTCT	CTATTCTGAG	GCTCCTGCGG	C.TGCCGCGC.	TGACTTCCC.
aa159394	GCAGTTCTCT	CTATTCTGAG	GCTCCTGCGG	CNTGCCNGCG	TGACTTCCCC
aa369714	GCAGTTCTNT	CTATTCTGAG	GCTCCTNCGG	C.TGCCGCGC	TGACTTCCC.
	151				200
consensus	TGTGTGGNGG	AGGGAACCTCT	GGGCAGGCTG	GTTTTCTTGG	AATGTGTTTA
Seq	TGTGTGCGGG	AGGGAACCTCT	GGGCAGGCTG	GTTTTCTTGG	AATGTGTTTA
thc117484	TGTGTGCGGG	AGGGAACCTCT	GGGCAGGCTG	GTTTTCTTGG	AATGTGTTTA
r65982	TGTGTGCGGG	AGGGAACCTCT	GGGCAGGCTG	GTTTTCTTGG	AATGTGTTTA
aa159394	TGTGTGGNGG	AGGGAACCTCT	GGGCAGGCTG	GTTTTCTTGG	AATGTGTTTA
aa369714	TGTGTGCGGG	AGGGAACCTCT	GGGCAGGCTG	GTTTTNTTGG	AATGTGTTTA
	201				250
consensus	CGAT.GTTGA	ATGGGACTTG	AACAGG..AA	GCTGGACGCT	GCA.GCTGGA
				primer oGA103	
		primer oGA104			
Seq	CGAT GTTGA	ATGGGACTTG	AACAGG AA	GCTGGACGCT	GCA GCTGGA
r65983rccCTTG	AAACGGGNAA	CCGGGCCNCT	GCAAGCNGGA
thc117484	CGAT.GTTGA	ATGGGACTTG	AACAGG..AA	GCTGGACGCT	GCA.GCTGGA
r65982	CGAT.GTTGA	ATGGGACTTG	AACAGG..AA	GCTGGACGCT	GCA.GCTGGA
aa159394	CGAT.GTTGA	ATGGGACTTG	AACAGG..AA	GCTGGACGCT	GCA.GCTGGA
aa369714	CGATTGTTGA	ATGGGACTTG	AACAGG..AA	GCTGGACGCT	GCA.....
	251				300
consensus	ACTAGCGTGC	C.AAGTTATT	TATGATTCC.	ATCTGATATA	CATAGGAGAG
Seq	ACTAGCGTGC	C AAGTTATT	TATGATTCC	ATCTGATATA	CATAGGAGAG
r65983rcc	ACTACCGTGC	CCAAGTTATT	TATGANCCCC	ACCTGATATA	CATGGGAGAG
thc117484	ACTAGCGTGC	C.AAGTTATT	TATGATTCC.	ATCTGATATA	CATAGGAGAG
r65982	ACTAGCGTGC	C.AAGTTATT	TATGATTCC.	ATCTGATATA	CATAGGAGAG
aa159394	ACTAGCGTGC	C.AAGTTATT	TATGATTCC.	ATCTGNTATA	CATAGGAGAG
	301				350
consensus	AAACT GATA	GAAGAATTCT	GATGGCAACT	GTATGATAG	AAGCTAT AT
				primer 445-10934-02F	
			primer oGA102		
Seq	AAACT GATA	GAAGAATTCT	GATGGCAACT	GTATGATAG	AAGCTAT AT
oGA102TA
r65983rcc	AAACT.GATA	GAAGAATTCT	GATGGCAACT	.GTATGATAG	AAGCTAT.AT
thc117484	AAACT.GATA	GAAGAATTCT	GATGGCAACT	.GTATGATAG	AAGCTAT.AT
r65982	AAACT.GATA	GAAGAATTCT	GATGGCAACT	.GTATGATAG	AAGCTAT.AT
aa159394	AAACTTGATA	GAAGAATTCT	GATGGCAACT	.GTATGATAG	AAGCTAT.AT

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FIG. 1 (CONTINUED)

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	351		400
consensus	AAAGTCAAGT GTCCATTTTC	TTTCAACTAT ATTTGAGCAT	ACCCAGGATT
Seq	AAAGTCAAGT GTCCATTTTC	TTTCAACTAT ATTTGAGCAT	ACCCAGGATT
oGA102	CAAGTCA.GT GTCCATTTTC	TTTCAACTAT ATTTGAGCAT	ACCCAGGATT
r65983rcc	AAAGTCAAGT GTCCATTTTC	TTTCAACTAT ATTTGAGCAT	ACCCAGGATT
thc117484	AAAGTCAAGT GTCCATTTTC	TTTCAACTAT ATTTGAGCAT	ACCCAGGGTT
r65982	AAAGTCAAGT GTCCATTTTC	TTTCAACTAT ATTTGAGCAT	ACCCAGGGTT
aa159394	AAAGTCAAGT GTCCATTTTC	TTTCAACTAT ATTTGAGCAT	ACCCAGGATT
hCED-6			M N R
	401		450
consensus	TAAGTCGTGG AACTGAACAT	TTATTTGGCT GATCCTCATC	ATG.AACCGT
		Primer 445-10934-07-R	
Seq	TAAGTCGTGG AACTGAACAT	TTATTTGGCT GATCCTCATC	ATG AACCGT
oGA102	TAAGTCGTGG AACTGAACAT	TTATTTGGCT GATCCTCATC	ATG.AACCGT
r65983rcc	TAAGTCGTGG AACTGAACAT	TTATTTGGCT GATCCTCATC	ATG.AACCGT
thc117484	TAAGTCGTGG AACTGAACAT	TTATTTGGCT GATCCTCATC	ATGGAACCGT
r65982	TAAGTCGTGG AACTGAACAT	TTATTTGGCT GATCCTCATC	ATGGAACCGT
aa159394	TAAGTCGTGG AACTGAACAT	TAT.....
CED-6	MAKDIYKTFK RSVSGIVGGN	NINGEGSSSP STSAPQVKYR	GGTG ...
CED-6		R T W I H P	P D Y L
hCED-6	A F S R K K D	K T W M H T	P E A L
	451		500
consensus	GCTTTTAGCA GGAAGAAAGA	CAAAACATGG ATGCATACAC	CTGAAGCTTT
Seq	GCTTTTAGCA GGAAGAAAGA	CAAAACATGG ATGCATACAC	CTGAAGCTTT
oGA102	GCTTTTAGCA GGAAGAAAGA	CAAAACATGG ATGCATACAC	CTGAAGCTTT
r65983rcc	GCTTTTAGCA GGAAGAAAGA	CAAAACATGG ATGCATACAC	CTGAAGCTTT
thc117484	GCTTTTAGCA GGAAGAAAGA	CAAAACATGG GTGCTNACAC	CTGAAG.NTT
r65982	GCTTTTAGCA GGAAGAAAGA	CAAAACATGG GTGCTNACAC	CTGAAG.NTT
CED-6	I N G H V E	Y V A R F L G	C V E
hCED-6	S K H F I P	Y N A K F L G	S T E
	501		550
consensus	ATCAAAACAT TTCATTCCCT	ATAATGCAAA GTTTCTTGGC	AGTACAGAAG
Seq	ATCAAAACAT TTCATTCCCT	ATAATGCAAA GTTTCTTGGC	AGTACAGAAG
oGA102	ATCAAAACAT TTCATTCCCT	ATAATGCAAA GTTTCTTGGC	AGTACAGAAG
r65983rcc	ATCAAAACAT TTCATTCCCT	ATAATGCAAA GTTTCTTGGC	AGTACAGAAG
thc117484	ATCAAAAC.N TTCTTTCCNA	TTT.....
r65982	ATCAAAAC.N TTCTTTCCNA	TTT.....
CED-6	T P K A N G S	D V A R E A I	H A I
hCED-6	V E Q P K G T	E V V R D A V	R K L
	551		600
consensus	TGGAACAGCC AAAAGGAACA	GAAGTTGTGA GAGATGCTGT	AAGGAAACTA
Seq	TGGAACAGCC AAAAGGAACA	GAAGTTGTGA GAGATGCTGT	AAGGAAACTA
oGA102	TGGAACAGCC AAAAGGAACA	GAAGTTGTGA GAGATGCTGT	AAGGAAACTA
r65983rcc	TGGAACAGCC AAAAGGAACA	GAAGTTGTGA GAGATGCTGT	AAGGAAACTA
CED-6	R F Q R D L K	R S E QTRETAK	L Q K V
hCED-6	K F A R H I K	K S E G Q K	I P K V
	601		650
consensus	AAGTTTGCAA GACATATCAA	GAAATCTGAA GGCCAGAAAA	TTCCTAAAGT
Seq	AAGTTTGCAA GACATATCAA	GAAATCTGAA GGCCAGAAAA	TTCCTAAAGT
oGA102	AAGTTTGCAA GACATATCAA	GAAATCTGAA GGCCAGAAAA	TTCCTAAAGT
r65983rcc	AAGTTTGCAA GACATNTCAA	GAAATCTGAA GGCCAAAAAA	AAAAAAAAG.
r76378	.AGTTTGCAA GACATATCAA	GAAATCTGAA GGCCAGAAAA	TTCCTAAAGT

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FIG. 1. (CONTINUED)

CED-6		E	I	R	I	S	I	D	N	V	I	I	A	D	I	K	T		
hCED-6		E	L	Q	I	S	I	Y	G	V	K	I	L	E	P	K	T		
		651															700		
	consensus	GGAGTTGCAA	ATATCAATTT	ATGGAGTAAA	AATTCTAGAA	CCCAAAACAA													
	Seq	GGAGTTGCAA	ATATCAATTT	ATGGAGTAAA	AATTCTAGAA	CCCAAAACAA													
	oGA102	GGAGTTGCAA	ATATCAATTT	ATGGAGTAAA	AATTCTAGAA	CCCAAAACAA													
	r76378	GGAGTTGCAA	ATATCAATTT	ATGGAGTAAA	AATTCTAGAA	CCCAAAACAA													
CED-6		K	A	P	M	Y	T	F	P	L	G	R	I	S	F	C	A	D	
hCED-6		K	E	V	Q	H	N	C	Q	L	H	R	I	S	F	C	A	D	
		701																750	
	consensus	AGGAAGTTCA	ACACAATTGC	CAGCTTCATA	GAATATCTTT	TTGTGCAGAT													
	Seq	AGG																	
	oGA102	AGG.....													
	r76378	AGGAAGTTCA	ACACAATTGC	CAGCTTCATA	GAATATCTTT	TTGTGCAGAT													
	d82787CAATTGC	CAGCTTCATAGNAATATCTTGGGNGTGCAGAT															
CED-6		D	K	D	D	K	R	M	F	S	F	I	A	R	A	E	G	A	S
hCED-6		D	K	T	D	K	R	I	F	T	F	I	C	K	D	S	E	S	
		751																	800
	consensus	GATAAAACTG	ACAAGAGGAT	ATTCAC TTTC	ATATGCAAAG	ATTCTGAGTC													
	r76378	GATAAAACTG	ACAAGAGGAT	ATTCAC TTTC	ATATGCAAAG	ATTCTGAGTC													
	d82787	GATAAAACTG	ACAAGAGGAT	ATTCAC TTTC	ATATGCAAAG	ATTCTGAGTC													
CED-6		G	K	P	S	C	Y	A	F	T	S	E	K	L	A	E	D		
hCED-6		N	K	H	L	C	Y	V	F	D	S	E	K	C	A	E	E		
		801																	850
	consensus	AAATAAACAT	TTGTGCTATG	TATTTGACAG	CGAAAAGTGT	GCTGAAGAGA													
	Seq					CTGAAGAGA													
	oGA102	CTGAAGAGA													
	r76378	AAATAAACAT	TTGTGCTATG	TATTTGACAG	CGAAAAGTGT	GTAAGTATCC													
	aa307982	GT GCTGAAGAGA													
	d82787	AAATAAACAT	TTGTGCTATG	TATTTGACAG	CGNAAAAGTGTGCTGAAGAGA														
CED-6		I	T	L	T	I	G	E	A	F	D	L	A	Y	K	R	F		
hCED-6		I	T	L	T	I	G	Q	A	F	D	L	A	Y	R	K	F		
		851																	900
	consensus	TCACTTTAAC	AATTGGCCAA	GCATT TGA..	CCTGGCATA C	AGGAAATTTC													
	pGA101					TC													
	Seq	TCACTTTAAC	AATTGGCCAA	GCATT TGA	CCTGGCATA C	AGGAAATTTC													
	oGA102	TCACTTTAAC	AATTGGCCAA	GCATT TGA..	CCTGGCATA C	AGGAAATTTC													
	r76378	CAGATGTTGT	AGGGGTGTT	TGTTCTGTTT	TATAAGNCC	GGGGATTGTC													
	aa307982	TCACTTTAAC	AATTGGCCAA	GCATT TGA..	CCTGGCATA C	AGGAAATTTC													
	d82787	TCACTTTAAC	AATTGGCCAA	GCATT TGN	CTGGCATA C	AGGAAATTTC													
CED-6		L	D	K	N	R	T	S	L	E	N	Q	K		Q	I	Y	I	
hCED-6		L	E	S	G	G	K	D	V	E	T	R	K		Q	I	A	G	
		901																	950
	consensus	TAGAA.TCAG	GAGGAAAAGA	TGTTGAAACA	AGAAAA...C	AGATCGCAGG													
						Primer oGA107-F													
						Primer 445-10934-04													
						Primer 445-10934-08-R													
	pGA101	TAGAA TCAG	GAGGAAAAGA	TGTTGAAACA	AGAAAA	C AGATCGCAGG													
	Seq	TAGAA TCAG	GAGGAAAAGA	TGTTGAAACA	AGAAAA	C AGATCGCAGG													
	oGA102	TAGAA.TCAG	GAGGAAAAGA	TGTTGAAACA	AGAAAA...C	AGATCGCAGG													
	aa307982	TAGAA.TCAG	GAGGAAAAGA	TGTTGAAACA	AGAAAA...C	AGATCGCAGG													
	d82787	TNGAA.TCAG	GAGGAAAAGA	TGTTGAAACA	AGAAAA...C	AGATCGCAGG													

FIG. 1. (CONTINUED) 4/56

CED-6		L	K	K	K	I	V	E	L	E	T	E	N	Q	V	L	I	
hCED-6		L	Q	K	R	I	Q	D	L	E	T	E	N	M	E	L	K	
		951															1000	
	consensus	GTTACAAAAA	AGAATCCAAG	ACTTAGAAAC	AG	AAAATAT	GGA	AACTTAAA										
	pGA101	GTTACAAAAA	AGAATCCAAG	ACTTAGAAAC	AG	AAAATAT	GGA	AACTTAAA										
	Seq	GTTACAAAAA	AGAATCCAAG	ACTTAGAAAC	AG	AAAATAT	GGA	AACTTAAA										
	oGA107																AAA	
	oGA102	GTTACAAAAA	AGAATCCAAG	ACTTAGAAAC	AGG	AAAATAT	GGA	AACTTAAA										
	r76378	CTTG.....										
	aa307982	GTTACAAAAA	AGAATCCAAG	ACTTAGAAAC	AG	AAAATAT	GGA	AACTTAAA										
	d82787	GTTACAAAAA	AGACTCCANG	ACTTAGAAAC	AG	AAAATAT	GGT										
CED-6		I	E	R	L	A	E	A	L	R	A	N	S	K	A	D	Y	
hCED-6		N	K	V	Q	D	L	E	N	Q	L	R	I	T	Q	V	S	
		1001															1050	
	consensus	AATAAAGTAC	A	AGATTTGG	AAAACCAACT	GAGAATAACT	CAAGTATCAG											
	pGA101	AATAAAGTAC	A	AGATTTGG	AAAACCAACT	GAGAATAACT	CAAGTATCAG											
	Seq	AATAAAGTAC	A	AGATTTGG	AAAACCAACT	GAGAATAACT	CAAGTATCAG											
	oGA107	AATAAAGTAC	A	AGATTTGG	AAAACCAACT	GAGAATAACT	CAAGTATCAG											
	oGA102	AATAAAGTAC	A	AGATTTGG	AAAACCAACT	GANATAACT	CAAGTATCAG											
	aa307982	AATAAAGTAC	A	AGATTTGG	AAAACCAACT	GAGAATAACT	CAAGTATCAG											
CED-6		E	N	T	G	P	P	I	Y	P	G	L	G	P	P	A		
hCED-6		A	P	P	A	G	S	M	T	P	K	S	P	S	T	D		
		1051															1100	
	consensus	CACCTCCAGC	AGG	CA	GT	ATGACACCTA	AG	TCGCCC	TCCACT	GAC								
	pGA101	CACCTCCAGC	AGG	CA	GT	ATGACACCTA	AG	TCGCCC	TCCACT	GAC								
	Seq	CACCTCCAGC	AGG	CA	GT	ATGACACCTA	AG	TCGCCC	TCCACT	GAC								
	oGA107	CACCTCCAGC	AGG	CA	GT	ATGACACCTA	AG	TCGCCC	TCCACT	GAC								
	oGA102	CACCTCCAGC	AGGGCAA	GT	ATGACACCTT	AAGTTGCCC	TCCACTTGAC											
	aa307982	CACCTCCAGC	AGGCA	GT	ATGACACCTA	AG	TCGCCC	TCCACT	GAC									
CED-6	LPLSPM	P	Q	G	P	P	P	N	I	P	P	S	S	I	Y	S		
hCED-6		I	F	D	M	I	P	F	S	P	I	S	H	Q	S	S		
		1101															1150	
	consensus	ATCTTTGATA	TGATTCCAT	TTTC	TCCA	ATAT	CACAC	C	AGTCTTC									
	pGA101	ATCTTTGATA	TGATTCCAT	TTTC	TCCA	ATAT	CACAC	C	AGTCTTC									
	Seq	ATCTTTGATA	TGATTCCAT	TTTC	TCCA	ATAT	CACAC	C	AGTCTTC									
	oGA107	ATCTTTGATA	TGATTCCAT	TTTC	TCCA	ATAT	CACAC	C	AGTCTTC									
	oGA102	ATCTTTGATA	ATGATTCCCT	TTTCTTCCA	ATATTACAC	CCAGTATTCTN												
	aa307982	ATCTTTG	ATGATTCCAT	TTTCT	CC	AATATCACAC	C	AGTCTTC										
	aa443368		CCAT	TTTCT	CC	AATATCACA	CCAGTCTTC											
CED-6		M	P	R	A	N	D	L	P	P	T	E	M	A	P			
hCED-6		M	P	T	R	N	G	T	Q	P	P	P	V	P	S			
		1151															1200	
	consensus	GATGCCTAC	TCGCAAT	GGCACACAGC	C	ACCTC	CA	GTACCTAGTA										
	pGA101	GATGCCTAC	TCGCAAT	GGCACACAGC	C	ACCTC	CA	GTACCTAGTA										
	Seq	GATGCCTAC	TCGCAAT	GGCACACAGC	C	ACCTC	CA	GTACCTAGTA										
	oGA107	GATGCCTAC	TCGCAAT	GGCACACAGC	C	ACCTC	CA	GTACCTAGTA										
	oGA102	GATGCCTTCC	TTCGCAATTG	GNACCACAGC	CCACCTTNCA	GTTCTTAGT												
	aa307982	GATGCCTAC	T	CGCAAT	GGCACACAGC	C	ACCTC	CA	GTACCTAG									
	aa443368	GATGCCTAC	TCGCAAT	GGCACACAGC	C	ACCTC	CA	GTACCTAGTA										
	aa431995	CAGCAAGTC	AACATTTGAC	ATATAGTTAT	TTATTAGTTG												
CED-6		T	L	P	Q	I	S	T	S	S	N	G	A	S	P	S	V	S
hCED-6		R	S	T	E	I	K	R	D	L	F	G	A	E	P	F	D	P
		1201																1250
	consensus	GATCTACTGA	GATTAAACGG	GACCTGTTTG	GAGCAGAACC	TTTTGACCCA												
	pGA101	GATCTACTGA	GATTAAACGG	GACCTGTTTG	GAGCAGAACC	TTTTGACCCA												

FIG. 1 (CONTINUED) *5/56*

Seq	GATCTACTGA	GATTAAACGG	GACCTGTTTG	GAGCAGAACC	TTTTGACCCA
oGA107	GANCTACTGA	GATTAAANGG	GACCTGTTTG	GAGCAGAACC	TTTTGACCCA
oGA102	NANG.....
aa443368	GATCTACTGA	GATTAAACGG	GACCTGTTTG	GAGCAGAACC	TTTTGACCCA
aa431995	ATCAAAGCAT	GAATATTTCA	ACTTTAGTGT	TCACTGATTT	TATTTTGCTG
CED-6	P A S	T S P S	G P A	P S I	P P P A
hCED-6	F N C	G A A D	F P P	D I Q	S K L D
	1251				1300
consensus	TTTAACTGTG	GAGCAGCAGA	TTTCCCTCCA	GATATTCAAT	CAAAATTAGA
	<u>Primer oGA108-F</u>				
pGA101	TTTAACTGTG	GAGCAGCAGA	TTTCCCTCCA	GATATTCAAT	CAAAATTAGA
Seq	TTTAACTGTG	GAGCAGCAGA	TTTCCCTCCA	GATATTCAAT	CAWAATTAGA
oGA107	TTTAACTGTG	GAGCAGCAGA	TTTCCCTCCA	GATATTCAAT	CAAAATTAGA
aa443368	TTTAACTGTG	GAGCAGCAGA	TTTCCCTCCA	GATATTCAAT	CAAAATTAGA
aa431995	TAACATTT	CATAGTC	TTTTTTA CA	GATATTAATT	ATTTTATTCT
CED-6	S T S	P S G	P A P S	I P P	P R P
hCED-6	E M Q	E G F	K M G L	T L E	G T V
	1301				1350
consensus	TGAGATGCAG	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAGTAT
pGA101	TGAGATGCAG	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAGTAT
Seq	TGAGATGCAG	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAGTAT
oGA107	TGAGATGCAG	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAAGTAT
oGA108	G	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAGTAT
aa443368	TGAGATGCAG	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAGTAT
aa431995	GTTTTA.CAG	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAGTAT
CED-6	P A L A	P P P	P V A	...	
hCED-6	F C L D	P L D	S R C	*	
	1351				1400
consensus	TTTGTCTCGA	CCCGTTAGAC	AGTAGGTGCT	GACATCAAGA	ACAAGAAATC
	<u>primer 445-10934-11-F</u>				
Seq	TTTGTCTCGA	CCCGTTAGAC	AGTAGGTGCT	GACATCAAGA	ACAAGAAATC
oGA107	TTTGTCTCGA	CCCGTTAGAC	AGTAGGTGCT	GACATCAAGA	ACAAGAAATC
oGA108	TTTGTCTCGA	CCCGTTAGAC	AGTAGGTGCT	GACATCAAGA	ACAAGAAATC
aa443368	TTTGTCTCGA	CCCGTTAGAC	AGTAGGTGCT	GACATCAAGA	ACAAGAAATC
aa431995	TTTGTCTCGA	CCCGTTAGAC	AGTAGGTGCT	GACATCAAGA	ACAAGAAATC
CED-6	...PRRNPVVS PKNSTAGLLD GLELGS AEP A KKAPS NIFDD				
CED-6	SFDPRAGEKK STAAEYNPFG ADFLSGIQNG KEAPPSASAE LLASEAIARL PKPESSSVPP				
CED-6	KKTA AEYDAM INEVEKKLAA MSSGSFEFGQ LQTGDLGGIE GESDYGTSPD RLNP KMMNLKQ				
	1401				1450
consensus	CTGATTCATG	TTAAATGTGT	TTGTATAC	A CATGTCATTT	ATTATTATTA
	<u>primer oGA109-F</u>				
Seq	CTGATTCATG	TTAAATGTGT	TTGTATAC	A CATGTCATTT	ATTATTATTA
oGA107	CTGATTCATG	TTAAATGTGT	TTGTATAC	A CATGTCATTT	ATTATTATTA
oGA109				ACTGTTTCATT	ATTATTATT
oGA108	CTGATTCATG	TTAAATGTGT	TTGTATAC	A CATGTCATTT	ATTATTATTA
aa443368	CTGATTCATG	TTAAATGTGT	TTGTATAC	A CATGTCATTT	ATTATTATTA
aa431995	CTGATTCATG	TTAAATGTGT	TTGTATAC	A CATGTCATTT	ATTATTATTA
r33389	CTGATTCATG	TTAAATGTGT	TTGTATAC	A CATGTCATTT	ATTATTATTA
	1451				1500
consensus	CTTTAAGATA	GGTATTA	TT CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
Seq	CTTTAAGATA	GGTATTA	TT CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
oGA107	CTTTAAGATA	GGTATTA	TT CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
oGA109	CTTTANNA	GGTTATTATT	NTGCGNTCA	GNTTTTNTAA	TATTTTAATA
oGA108	CTTTAAGATA	GGTATTA	TT CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
aa443368	CTTTAAGATA	GGTATTA	TT CATGTGTCAA	TGTTTTTGAA	TATTTTAATA

FIG. 1. (CONTINUED) 6/56

aa431995	CTTTAAGATA	GGTATTA	TT	CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
r53881GATA
r62236AAGATA	GGTATTA	.TT	CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
h03749	...TAAGATA	GGTATTA	.TT	CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
r33389	CTTTAAGATA	GGTATTA	.TT	CATGTGTCAA	TGTTTTTGAA	TATTTTAATA
	1501					1550
consensus	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT....T	CACTATTGAT	
Seq	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT	T	CACTATTGAT
oGA109	TTTNTAAAAT	TTTCTCANTT	AAATTTTCCT	CACCT	T	CACTATTNNAT
oGA108	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT	T	CACTATTGAT
aa443368	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT	T	CACTATTGAT
aa431995	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT	T	CACTATTGAT
r53881	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT....T	CACTATTGAT	
r62236	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT....T	CACTATTGAT	
h03749	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT....T	CACTATTGAT	
r33389	TTTTGAAAAT	TTTCTCAGTT	AAATTTTCCT	CACCT....T	CACTATTGAT	
	1551					1600
consensus	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G...TAAAGT	AGA..TCATA	
				<u>primer 445-10934-03-R</u>		
Seq	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G	TAAAGT	AGA TCATA
oGA109	CGTTAATTTT	TATTTTAAAA	ACNTCTTACN	T	TAANTT	NNA TCATA
oGA108	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G	TAAAGT	AGA TCATA
aa443368	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	GT		
aa431995	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G	TAAAGT	AG A TCATA
r53881	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G...TAAAGT	AG A TCATA	
r62236	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G...TAAAGT	AG A TCATA	
h03749	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G...TAAAGT	AG A TCATA	
r33389	CTGTAATTTT	TATTTTAAAA	ACAGCTTACT	G...TAAAGT	AGGA TCATA	
	1601					1650
consensus	CTTTT..ATG	TTCCTTTCTG	TTTCTACTGT	AGAT..GAAT	TTGTAATTGA	
Seq	CTTTT ATG	TTCCTTTCTG	TTTCTACTGT	AGAT	GAAT	TTGTAATTGA
oGA109	CTTTT ANN	TTCCTTTCTGA	TTTCTACGC	TNNA	GNA	TTGNTAATTATA
oGA108	CTTTT ATG	TTCCTTTCTG	TTTCTACTGT	AGAT	GAAT	TTGTAATTGA
aa431995	CTTTT ATG	TTCCTTTCTG	TTTCTACTGT	AGAT	GAAT	TTGTAATTGA
r53881	CTTTT..ATG	TTCCTTTCTG	TTTCTACTGT	AGAT..GAAT	TTGTAATTGA	
r62236	CTTTT..ATG	TTCCTTTCTG	TTTCTACTGT	AGAT..GAAT	TTGTAATTGA	
h03749	CTTTT..ATG	TTCCTTTCTG	TTTCTACTGT	AGAT..GAAT	TTGTAATTGA	
r33389	CTTTT..ATG	TTCCTTTCTG	TTTCTACTGT	AGGATGGAAT	TTGTAATTGG	
	1651					1700
consensus	AAG.ACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAG.TT	CTATTTAGTT	
				<u>primer 445-10934-06-F</u>		
Seq	AAG ACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAG	TT	CTATTTAGTT
oGA109	ANT ACATAT	TATACAAATA	CCGACCTTANGAT	CTNNNTTT	CTATTTATTT	
oGA108	AAG ACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAG	TT	CTATTTAGTT
aa431995	AAG ACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAG	TT	C
r53881	AAG.ACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAG.TT	CTATTTAGTT	
r62236	AAG.ACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAG.TT	CTATTTAGTT	
h03749	AAG.ACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAG.TT	CTATTTAGTT	
r33389	AAGACATAT	TATACAAATA	CCTGCCTTGT	GTCTGAGGTT	CTATTAGGTA	
	1701					1750
consensus	AGC.ATCTTG	AAATTTGTAT	TCATTTTCCA	GATGGCTAGT	TTATTAATGA	
				<u>Primer oGA110-F</u>		
Seq	AGC ATCTTG	NAATTTGTAT	TCATTTTCCAGGAT	GATGGCTAGT	TTATTAATGA	
oGA109	NTC ATCTGT	AAATTGATAT	TCATTTTCCA	TAGGNCTGTTT	TATTAAGNAT	
oGA108	AGC ATCTTG	AAATTTGTAT	TCATTTTCCA	GATGGCTAGT	TTATTAATGA	
oGA110					CTTTAATGA	

FIG. 1. (CONTINUED) 7/56

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r53881 AGC.ATCTTG AAATTTGTAT TCATTTTCCA GATGGCTAGT TTATTAATGA
r62236 AGC.ATCTTG AAATTTGTAT TCATTTTCCA GATGGCTAGT TTATTAATGA
h03749 AGC.ATCTTG AAATTTGTAT TCATTTTCCA GATGGCTAGT TTATTAATGA
r33389 GGCCATCTGG AAATTTGTAT TCATT.....

1751 1800
consensus TTTCCCAAAA GCCATACCTT AAAG.ATAAC TTTTTAAATT CTGAAGA..G
primer 445-10934-12-R
Seq TTTCCCAAAA GCCATACCTT AAAG ATAAC TTTTTAAATT CTGAAGA G
oGA109 TTCCCAAAN TCCATACCTT AANT ATAAC TTTTTAAATT TTTAATA T
oGA108 TTTCCCAAAA GCCATACCTT AAAG ATAAC TTTTTAAATT CTGAAGA G
oGA110 TTTCCCAAAA GCCATACCTT AAAG ATAAC TTTTTAAATT CTGAAGA G
r53881 TTTCCCAAAA GCCATACCTT AAAG.ATAAC TTTTTAAATT CTGAAGG..G
r62236 TTTCCCAAAA GCCATACCTT AAAG.ATAAC TTTTTAAATT CTGGAAGA.G
h03749 TTTCCCAAAA GCCATACCTT AAAGGATAAC TTTTTAAATT CTGGAAGGNG

1801 1850
consensus ACATGCCAAT GTCAAACCTAA ACATGTTCTG TTTTTAAA.C CAACAAACAT
Seq ACATGCCAAT GTCAAACCTAA ACATGTTCTG TTTTTAAA C CAACAAACAT
oGA109 ACANTCCAA GTTCAAACCTAA ACANNITCGN TTTTTAAA C CAACAAACAN
oGA110 ACATGCCAAT GTCAAACCTAA ACATGTTCTG TTTTTAAA C CAACAAACAT
oGA108 ACATGCCAAT GTCAAACCTAA ACATGTTCTG TTTTTAAA C CAACAAACAT
r53881 ACATGCCAAT GTCAAACCTAA ACATGTTCTG TTTTTAAA.C CAACAAACAT
r62236 ACATGCCAAT GTCAAACCTAA ACATGTTCTG TTTTTAAAAC CAACAAACAT
h03749 ACATGCCAAT GTCAAACCTAA ACATGTTCCG TTTTTAAAAC CAACAAACAT

1851 1900
consensus GTTA.CTATT CATTGG.ACA GATATCATT TATG..TATA AATACTGTT.
Seq GTTA CTATT CATTGG ACA GATATCATT TATG TATA AATACTGTT
oGA109 NTTA.CTATT CATGNGNACA NATATCATT TANA TATA AACACTANT
oGA108 GTTA CTATT CATTGG ACA GATATCATTN NATG TATA AAT
oGA110 GTTA CTATT CATTGG ACA GATATCATT TATG TATA AATACTGTT
r53881 GTTA.CTATT CATTGGGACA GNTATCCTT TATGGTATTA AATACTGTTC
r62236 GTTAACTATT TCATGGGACA .....
h03749 GTTA.CTATT TCATG.....

1901 1950
consensus CACATCACTG G.GAAAATGT AAACCTT.AA ACATAATGCC ACAAGGTCAC
Seq CACATCACTG G GAAAATGT AAACCTT AA ACATAATGCC ACAAGGTCAC
oGA109 TCACATCACTG GGTAAGAT AANCTT AA ACATAATACCCACANGTTCAC
oGA110 CACATCACTG G GAAAATGT AAACCTT AA ACATAATGCC ACAAGGTCAC
r53881 CACCTCACCG GGGGNATGGT AAACCTTNAA ACCTNATGGC CNCAGGGGCA

1951 2000
consensus TAATTTCTAG CAGGTAAAT TATAAGGATA TAAATTCCAA TAATAAACCA
Seq TAATTTCTAG CAGGTAAAT TATAAGGATA TAAATTCCAA TAATAAACCA
oGA109 TAATTTCTAA CNGATNAAAT TATANGGNTATAAATTCCAA TAATAAACCA
oGA110 TAATTTCTAG CAGGTAAAT TATAAGGATA TAAATTCCAA TAATAAACCA
aa431753rcc .....GGTAAAT TATAAGGATA TAAATTCCAA TAATAAACCA
r53881 CCNTTTTNCG GCG.....

2001 2050
consensus AATGTATTTA GAGTATTTAT TAGTAAATGC AAGGTGATGT TAGTTATGAT
primer 445-10934-01 R
primer 445-10934-10-F
pGA101 AGTAAATGCCAAGGTGATGGTTAGTTAAGGAT
Seq AATGTATTTA GAGTATTTAT TAGTAAATGC AAGGTGATGT TAGTTATGAT
oGA109 AAGATATTTAAGAATATTTTANTAACTGC CAGNTGAA
oGA110 AATGTATTTA GAGTATTTAT TAGTAAATGC AAGGTGATGT TAGTTATGAT
aa431753rcc AATGTATTTA GAGTATTTAT TAGTAAATGC AAGGTGATGT TAGTTATGAT
aa159297rcc .....GATGAT

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FIG. 1. (CONTINUED)

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	2051		2100
consensus	CAGTTATACT CTAAATATTT AATTTGTTTT ATAAAGGTAG TGAAAAAATG		
pGA101	CAGGTTAAAACCTCTAAATATTNAATNTTGT	TTT	GAAAAAATG
Seq	CAGTTATACT CTAAATATTT AATTTGTTTT ATAAAGGTAG TGAAAAAATG		
oGA110	CAGTTATACT CTAAATATTT AATTTGTTTT ATAAAGGTAG TGAAAAAATG		
aa431753rcc	CAGTTATACT CTAAATATTT AATTTGTTTT ATAAAGGTAG TGAAAAAATG		
aa159297rcc	CAGTTATACT CNAATATTN AATTTGTNTT ATAAAGGTAG TGAAAAAATG		
aa770228rccTATACT CTAAATATTT AATTTGTTTT ATAAAGGTAG TGAAAAAATG		
h02853rccT	ATAAGGGTAG	NGAAAAAANG

	2101		2150
consensus	AAAATTTGCT ATTTATTAAA AAACATTAAA	TTTC.ATTCC	.AAATGAGAT
		primer 445-10934-05-F	
pGA101	AAAATTTGCT ATTTATTAAA AAACATTAAA	TTTC ATTCC	AAATGAGAT
Seq	AAAATTTGCT ATTTATTAAA AAACATTAAA	TTTC ATTCC	AAATGAGAT
oGA110	AAAATTTGCT ATTTATTAAA AAACATTAAA	TTTC ATTCC	AAATGAGAT
aa431753rcc	AAAATTTGCT ATTTATTAAA AAACATTAAA	TTTC.ATTCC	.AAATGAGAT
aa159297rcc	AAAATTTGCT ATTTATTAAA AAACATTAAA	TTTC.ATTCC	.AAATGAGAT
aa770228rcc	AAAATTTGCT ATTTATTAAA AAACATTGAA	TTTC.ATTCC	.AAATGAGAT
h02853rcc	AAAATTTGCT ATTTATTAAA AAACATTAAA	TTTC.ATTCC	CAAATGAGAT
d60819rccATTATKRAA	AAACATTAAA	TKTC.ATBCS .AAATGAGAT
r62135rccAAACATTAAA	TGTCCANGCC	CAAATGAGAT

	2151		2200
consensus	AAGTG.ATAT TAC.TATAAC ATC.TAAGCA TCATCT..GA	TTTG.ATATT	
pGA101	AAGTG ATAT TAC TATAAC ATC TAAGCA TCATCT GA	TTTG ATATT	
Seq	AAGTG ATAT TAC TATAAC ATC TAAGCA TCATCT GA	TTTG ATATT	
oGA110	AAGTG ATAT TAC TATAAC ATC TAAGCA TCATCT GA	TTTG ATATT	
aa431753rcc	AAGTG.ATAT TAC.TATAAC ATC.TAAGCA TCATCT..GA	TTTG.ATATT	
aa159297rcc	AAGTG.ATAT TAC.TATAAC ATC.TAAGCA TCATCT..GA	TTTG.ATATT	
aa770228rcc	AAGTG.ATAT TAC.TATAAC ATC.TAAGCA TCATCT..GA	TTTG.ATATT	
h02853rcc	AAGTG.ATAT TACCTATAAC ATCCTAAGCA TCATCT..GA	TTTG.ATANT	
d60819rcc	AAGTG.ATAT TAC.TATAAC ATC.TAAGCA TCATCT..GA	TTTG.ATATY	
r62135rcc	AAGTGGATAN TACCTATAAC ATCCTAAGCA TCATCCTGNA	TTTGNANANT	

	2201		2250
consensus	CCCT.AAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
pGA101	CCCT AAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
Seq	CCCT AAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
oGA110	CCCT AAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
aa431753rcc	CCCT.AAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
aa159297rcc	CCCT.NAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
aa770228rcc	CCCT.AAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
h02853rcc	CCCT.AAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	
d60819rcc	CCCTRAAAAA ASATKTGGA TATATGCTAT CTATAGAKTC	AGTATCTACT	
r62135rcc	CCCCTNAAAA ACATTGGA TATATGCTAT CTATAGATTC	AGTATCTACT	

	2251		2300
consensus	ACCCATATTT ACTTTACC.A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
pGA101	ACCCATATTT ACTTTACC A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
Seq	ACCCATATTT ACTTTACC A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
oGA110	ACCCATATTT ACTTTACC A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
aa431753rcc	ACCCATATTT ACTTTACC.A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
aa159297rcc	ACCCATATTT ACTTTACC.A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
aa770228rcc	ACCCATATTT ACTTTACC.A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
h02853rcc	ACCCATATTT ACTTTACC.A AATATATTTTC TCCTCACTGC	ATAAGGACTA	
d60819rcc	ACCCATATTT ACTTTACSSA AATATATTTTC TCCTCACTGC	ATAAGGACTA	
r62135rcc	ACCCATATTT ACTTTACC.A AATATATTTTC TCCTCACTGC	ATAAGGACTA	

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FIG. 1. (CONTINUED)

consensus	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
pGA101	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
Seq	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
oGA110	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
aa431753rcc	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
aa159297rcc	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
aa770228rcc	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
h02853rcc	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
d60819rcc	CTCTTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
r62135rcc	CTCNTCTCAT	ATTTTCTTCT	TTGATGAAGA	TATTTTTCAC	CAAAGTTTAT
	2351				2400
consensus	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
pGA101	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
Seq	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
oGA110	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
aa431753rcc	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
aa159297rcc	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
aa770228rcc	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
h02853rcc	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
d60819rcc	TTTGTGATGC	CCTCTTGGTT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
r62135rcc	TTTGTGATGC	CCTCTTGGNT	TTGATACTTT	AAAATCTGTG	GCACCCGTTT
	2401				2450
consensus	TACATGAATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
pGA101	TACATGAATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
Seq	TACATGAATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
oGA110	TACATGAATT	ATCAATATTT	GGTAA TTCA	ATCTGTATTT	GTTTTGGTAA
aa431753rcc	TACATGAATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
aa159297rcc	TACATGAATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
aa770228rcc	TACATGAATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
h02853rcc	TACATGAATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
d60819rcc	TACATGAATT	ATCAATATTT	GGTAAAKTCA	ATCTGTATTT	GTTTTGTTAA
r62135rcc	TACATGNATT	ATCAATATTT	GGTAAATTCA	ATCTGTATTT	GTTTTGTTAA
	2451				2498
consensus	AGTCAAAAAT	CTCATTTTCC
pGA101	AGTCAAAAAT	CTCATTTTCC	AGTCGACGCG	GCCGC	
Seq	AGTCAAAAAT	CTCATTTTCC	AAAAAAAAAA	AAAAAAAAACT	CGAG
oGA110	ATCCAAAAAT	NNNCATT			
aa431753rcc	AGTCAAAAAT	CTCATTTTCC	AAAA.....
aa159297rcc	AGTCAAAAAT	CTCATTTTCC
aa770228rcc	AGTCAAAAAT	CTCATTT...
h02853rcc	AGTCAAAAAN	NTCAANNTCC
d60819rcc	AGTCRAAAAT	CTCATTTTCC
r62135rcc	AGTNANNANT	CTCATTTTCC	AANANGGGGG	GGGGGGGGGA	AGTTCCTG

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FIG. 2.

GGTGATGAGCCCTTGGGTTCTCGCTCCGACTGCTAAATTCGCTTGGCCGGGTCCACCTTCTCGTGGCCT
 CACTCGCCACACGGATCAGAATCCGGAGCAGGCAGTTCTCTCTATTCTGAGGCTCCTGCGGCTGCCGCG
 CTGACTTCCCTGTGTGGNGGAGGGAAGTCTGGGCAGGCTGGTTTTCTTGGAATGTGTTTACGATGTTGA
 ATGGGACTTGAACAGGAAGCTGGACGCTGCAGCTGGAAGTACGCTGCCAAGTTATTTATGATTCCATC
 TGATATACATAGGAGAGAACTGATAGAAGAATTCTGATGGCAACTGTATGATAGAAGCTATATAAAG
 TCAAGTGTCCATTTTCTTTCAACTATATTTGAGCATAACCCAGGATTTAAGTCGTGGAAGTGAACATTTAT
 TTGGCTGATCCTCATCATGAACCGTGCTTTTAGCAGGAAGAAAGACAAAACATGGATGCATACACCTG
 AAGCTTTATCAAAACATTTTCATTCCCTATAATGCAAAGTTTCTTGGCAGTACAGAAGTGGAACAGCCAA
 AAGGAACAGAAGTTGTGAGAGATGCTGTAAGGAACTAAAGTTTGCAAGACATATCAAGAAATCTGA
 AGGCCAGAAAATTCCTAAAGTGGAGTTGCAAAATATCAATTTATGGAGTAAAAATTTCTAGAACCCAAAA
 CAAAGGAAGTTCAACACAATTGCCAGCTTCATAGAATATCTTTTTGTGCAGATGATAAACTGACAAG
AGGATATTCATTTTCATATGCAAAGATTCTGAGTCAAATAAACATTTGTGCTATGTATTTGACAGCGAA
AAGTGTGCTGAAGAGATCACTTTAACAATTGGCCAAGCATTTGACCTGGCATAACAGGAAATTTCTAGA
 ATCAGGAGGAAAAAGATGTTGAAACAAGAAAACAGATCGCAGGGTTACAAAAAAGAATCCAAGACTTA
 GAAACAGAAAATATGGAACCTTAAAAATAAAGTACAAGATTTGGAAAACCAACTGAGAATAACTCAAG
 TATCAGCACCTCCAGCAGGCAGTATGACACCTAAGTCGCCCTCCACTGACATCTTTGATATGATTCCAT
 TTTCTCCAATATCACACCAGTCTTCGATGCCTACTCGCAATGGCACACAGCCACCTCCAGTACCTAGTA
 GATCTACTGAGATTAAACGGGACCTGTTTGGAGCAGAACCTTTTGACCCATTTAACTGTGGAGCAGCA
 GATTTCCCTCCAGATATTCAATCAAAATTAGATGAGATGCAGGAGGGGTTCAAAATGGGACTAACTCT
 TGAAGGCACAGTATTTTGTCTCGACCCGTTAGACAGTAGGTGCTGACATCAAGAACAAGAAATCCTGA
 TTCATGTTAAATGTGTTTGTATACACATGTCATTTATTATTACTTTAAGATAGGTATTATTATCATGTG
 TCAATGTTTTTGAATATTTTAATATTTTGAATAATTTCTCAGTTAAATTTCCCTCACCTTCACTATTGATCT
 GTAATTTTTATTTTAAAAACAGCTTACTGTAAAGTAGATCATACTTTTATGTTTCTTTCTGTTTCTACTGT
 AGATGAATTTGTAATTGAAAGACATATTATACAAATACCTGCCTTGTGTCTGAGTTCTATTTAGTTAGC
 ATCTTGAAATTTGTATTCAATTTTCCAGATGGCTAGTTTATTAATGATTTCCCAAAAGCCATACCTTAAAG
 ATAACCTTTTAAATTCTGAAGAGACATGCCAATGTCAAATAAACATGTTCTGTTTTTAAACCAACAAA
 CATGTTACTATTCAATTGGACAGATATCATTTTATGTATAAATACTGTTACATCACTGGGAAAATGTAA
 ACTTTAAACATAATGCCACAAGGTCATAATTTCTAGCAGGTAAAATTATAAGGATATAAATTCCAATA
 ATAAACCAAAATGTATTTAGAGTATTTATTAGTAAATGCAAGGTGATGTTAGTTATGATCAGTTATACTC
 TAAATATTTAATTTGTTTTATAAAGGTAGTGAAAAAATGAAAATTTGCTATTTATTAATAAACATTAAA
 TTTCAATCCAAATGAGATAAGTGATATTACTATAACATCTAAGCATCATCTGATTTGATATTCCCTAAA
 AAACATTTGGAATATATGCTATCTATAGATTCAGTATCTACTACCCATATTTACTTTACCAAATATATTT
 CTCCTCACTGCATAAGGACTACTCTTCTCATATTTTCTTCTTTGATGAAGATATTTTTCACCAAAGTTTA
 TTTTGTGATGCCCTCTTGGTTTTGATACTTTAAATCTGTGGCACCCGTTCTACATGAATTATCAATATT
 TGGTAAATTCAATCTGTATTTGTTTTGTTAAAGTCAAAAATCTCATTTTCCAAAAAATAAAAAAAAAA
 CTCGAG

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FIG. 3.

GGTGATGAGCCCTTGGGTTCTCGCTCCGACTGCTAAATTCGCTTGGCCGGGTCCACCTTCTCGTGGCCT
CACTCGCCACACGGATCAGAATCCGGAGCAGGCAGTTCTCTCTATTCTGAGGCTCCTGCGGCTGCCGCG
CTGACTTCCCTGTGTGGNGGAGGGAACTCTGGGCAGGCTGGTTTTCTTGGAATGTGTTTACGATGTTGA
ATGGGACTTGAACAGGAAGCTGGACGCTGCAGCTGGAAGTAGCGTGCCAAGTTATTTATGATTCCATC
TGATATACATAGGAGAGAACTGATAGAAGAATTCTGATGGCAACTGTATGATAGAAGCTATATAAAG
TCAAGTGTCCATTTTCTTTCAACTATATTTGAGCATACCCAGGATTTAAGTCGTGGAAGTGAACATTTAT
TTGGCTGATCCTCATCATGAACCGTGTCTTTAGCAGGAAGAAAGACAAAACATGGATGCATACACCTG
AAGCTTTATCAAAACATTTTCAATTCCTATAATGCAAAGTTTCTTGGCAGTACAGAAGTGGAAACAGCCAA
AAGGAACAGAAAGTTGTGAGAGATGCTGTAAGGAACTAAAGTTTGCAAGACATATCAAGAAATCTGA
AGGCCAGAAAATTCCTAAAGTGGAGTTGCAAATATCAATTTATGGAGTAAAAATTCTAGAACCCAAAA
CAAAGGCTGAAGAGATCACTTTAACAATTGGCCAAGCATTGACCTGGCATAACAGGAAATTTCTAGAA
TCAGGAGGAAAAGATGTTGAAACAAGAAAACAGATCGCAGGGTTACAAAAAAGAATCCAAGACTTAG
AAACAGAAAATATGGAACCTTAAAAATAAAGTACAAGATTTGGAACCAACTGAGAATAACTCAAGT
ATCAGCACCTCCAGCAGGCAGTATGACACCTAAGTCGCCCTCCACTGACATCTTTGATATGATTCCATT
TTCTCCAATATCACACCAGTCTTCGATGCCTACTCGCAATGGCACACAGCCACCTCCAGTACCTAGTAG
ATCTACTGAGATTAAACGGGACCTGTTTGGAGCAGAACCTTTTGACCCATTTAACTGTGGAGCAGCAG
ATTTCCCTCCAGATATTCAATCAAAATTAGATGAGATGCAGGAGGGGTTCAAAATGGGACTAACTCTT
GAAGGCACAGTATTTGTCTCGACCCGTTAGACAGTAGGTGCTGACATCAAGAACAAGAAATCCTGAT
TCATGTTAAATGTGTTTGTATACACATGTCATTTATTATTACTTTAAGATAGGTATTATTCATGTGT
CAATGTTTTTGAATATTTTAATATTTTGAAAATTTTCTCAGTTAAATTTCTCACCTTCACTATTGATCTG
TAATTTTTATTTTAAAAACAGCTTACTGTAAAGTAGATCATACTTTTATGTTTCTTTCTGTTTCTACTGTA
GATGAATTTGTAATTGAAAGACATATTATACAAATACCTGCCTTGTGTCTGAGTTCTATTTAGTTAGCA
TCTTGAAATTTGTATTTCATTTTCCAGATGGCTAGTTTATTAATGATTTCCCAAAGCCATACCTTAAAGA
TAACTTTTTAAATTCTGAAGAGACATGCCAATGTCAAACATAACATGTTCTGTTTTTAAACCAACAAAC
ATGTTACTATTTCATTGGACAGATATCATTTTATGTATAAATACTGTTTACATCACTGGGAAAATGTAAA
CTTTAAACATAATGCCACAAGGTCATAATTTCTAGCAGGTAAAATTATAAGGATATAAATTCCAATAA
TAAACCAAATGTATTTAGAGTATTTATTAGTAAATGCAAGGTGATGTTAGTTATGATCAGTTATACTCT
AAATATTTAATTTGTTTTATAAAGGTAGTGAAAAAATGAAAATTTGCTATTTATTAAAAAACATTAAAT
TTCATTCCAAATGAGATAAGTGATATTACTATAACATCTAAGCATCATCTGATTTGATATTCCCTAAAA
AACATTTGGAATATATGCTATCTATAGATTCAGTATCTACTACCCATATTTACTTTACCAAATATATTTT
TCCTCACTGCATAAGGACTACTCTTCTCATATTTTCTTCTTTGATGAAGATATTTTTCACCAAAGTTTAT
TTTGTGATGCCCTCTTGGTTTTGATACTTTAAAATCTGTGGCACCCGTTCTACATGAATTATCAATATTT
GGTAAATTCAATCTGTATTTGTTTTGTAAAGTCAAAAATCTCATTTTCCAAAAAATAAAAAAAAAAAC
TCGAG

*12/56**FIG. 4.*

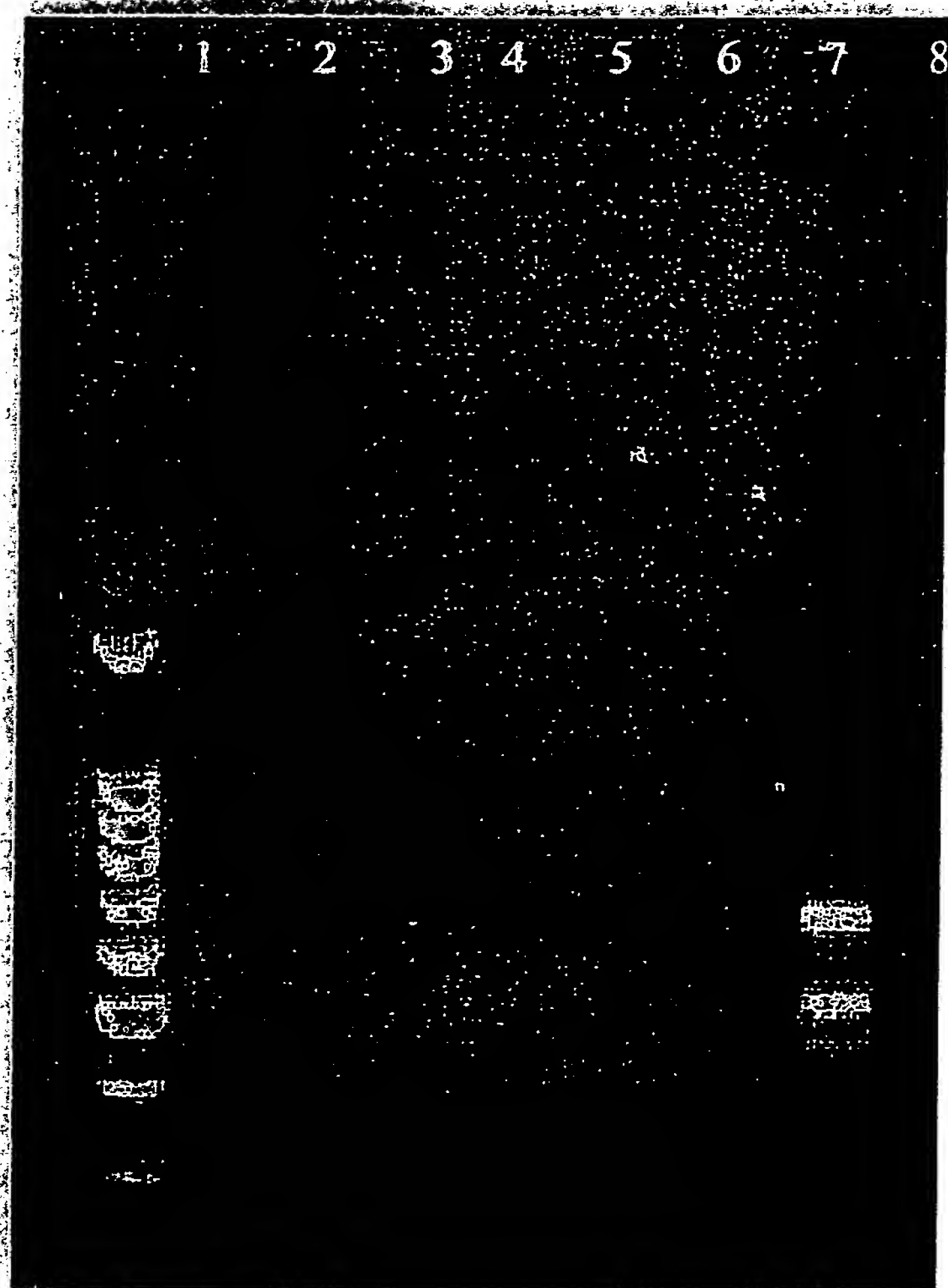
MNRAFSRKKDKTWMHTPEALSKHFIPYNAKFLGSTEVEQPKGTEVVRDAVRKLK FARHIKKS
EGQKIPKVELQISYGVKILEPKTKEVQHNCOLHRISFCADDKTDKRIFTFICKDSES NKHLCYV
FDSEKCAEEITLTIGQAFDLAYRK FLES GGKDVETRKQIAGLQKRIQDLETENMELKNKVQDLE
NQLRITQVSAPPAGS MTPKSPSTDIFDMIPFSPISHQSSMPTRNGTQPPVP SRSTEIKRDLFGAEP
FDPFNCGAADFPPDIQSKLDEM QEGFKMGLTLEGT VFCLDPLDSRC*

FIG. 5.

MNRAFSRKKDKTWMHTPEALSKHFIPYNAKFLGSTEVEQPKGTEVVRDAVRKLK FARHIKKS
EGQKIPKVELQISYGVKILEPKTKAEEITLTIGQAFDLAYRK FLES GGKDVETRKQIAGLQKRIQ
DLETENMELKNKVQDLENQLRITQVSAPPAGS MTPKSPSTDIFDMIPFSPISHQSSMPTRNGTQPP
PVPSRSTEIKRDLFGAEPDPFNCGAADFPPDIQSKLDEM QEGFKMGLTLEGT VFCLDPLDSRC*

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FIG. 6.



← Full length Ced-6 (700 bp)
← Splice variant Ced-6 (500 bp)
← Artefact (450 bp)

FIG. 7 14/56

SQ SEQUENCE 4729 BP

60 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG
120 CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC CCCGCCCAT
180 GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC ATTGACGTCA
240 ATGGGTGGAG TATTTACGGT AAAC TGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC
300 AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
360 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC
420 CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG
480 ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC AAAATCAACG
540 GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT
600 ACGGTGGGAG GTCTATATAA GCAGAGCTGG TTTAGTGAAC CGTCAGATCC GCTAGCGCTA
660 CCGGACTCAG ATCTCGAGCT CAAGCTTCGA ATTCTGCAGT CGACGGTACC GCGGGCCCGG
720 GATCCATCGC CACCATGGTG AGCAAGGGCG AGGAGCTGTT CACCGGGGTG GTGCCCATCC
780 TGGTCGAGCT GGACGGCGAC GTAAACGGCC ACAAGTTCAG CGTGTCCGGC GAGGGCGAGG
840 GCGATGCCAC CTACGGCAAG CTGACCCTGA AGTTCATCTG CACCACCGGC AAGCTGCCCC
900 TGCCCTGGCC CACCCTCGTG ACCACCCTGA CCTACGGCGT GCAGTGCTTC AGCCGCTACC
960 CCGACCACAT GAAGCAGCAC GACTTCTTCA AGTCCGCCAT GCCCGAAGGC TACGTCCAGG
1020 AGCGCACCAT CTTCTTCAAG GACGACGGCA ACTACAAGAC CCGCGCCGAG GTGAAGTTCC
1080 AGGGCGACAC CCTGGTGAAC CGCATCGAGC TGAAGGGCAT CGACTTCAAG GAGGACGGCA
1140 ACATCCTGGG GCACAAGCTG GAGTACAACT ACAACAGCCA CAACGTCTAT ATCATGGCCG
1200 ACAAGCAGAA GAACGGCATC AAGGTGAACT TCAAGATCCG CCACAACATC GAGGACGGCA
1260 GCGTGCAGCT CGCCGACCAC TACCAGCAGA ACACCCCAT CGGCGACGGC CCCGTGCTGC
1320 TGCCCGACAA CCACTACCTG AGCACCCAGT CCGCCCTGAG CAAAGACCCC AACGAGAAGC
1380 GCGATCACAT GGTCTTGCTG GAGTTCGTGA CCGCCGCCGG GATCACTCTC GGCATGGACG
1440 AGCTGTACAA GTAAAGCGGC CGCGACTCTA GATCATAATC AGCCATACCA CATTTGTAGA
1500 GGTTTTACTT GCTTTAAAAA ACCTCCCACA CCTCCCCCTG AACCTGAAAC ATAAAATGAA

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FIG. 7. (CONTINUED)

1560 TGCAATTGTT GTTGTTAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG
1620 CATCACAAAT TTCACAAATA AAGCATTTTT TCACTGCAT TCTAGTTGTG GTTGTCCAA
1680 ACTCATCAAT GATCTTAAG GCGTAAATTG TAAGCGTTAA TATTTTGTTA AAATTCGCGT
1740 TAAATTTTTG TTAAATCAGC TCATTTTTTA ACCAATAGGC CGAAATCGGC AAAATCCCTT
1800 ATAAATCAAA AGAATAGACC GAGATAGGGT TGAGTGTGT TCCAGTTTGG AACAAGAGTC
1860 CACTATTAAA GAACGTGGAC TCCAACGTCA AAGGGCGAAA AACCGTCTAT CAGGGCGATG
1920 GCCCACTACG TGAACCATCA CCCTAATCAA GTTTTTTGGG GTCGAGGTGC CGTAAAGCAC
1980 TAAATCGGAA CCCTAAAGGG AGCCCCCGAT TTAGAGCTTG ACGGGGAAAG CCGGCGAACG
2040 TGGCGAGAAA GGAAGGGAAG AAAGCGAAAG GAGCGGGCGC TAGGGCGCTG GCAAGTGTAG
2100 CGGTCACGCT GCGCGTAACC ACCACACCCG CCGCGCTTAA TCGCCGCTA CAGGGCGCGT
2160 CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC
2220 ATTCAAATAT GTATCCGCTC ATGAGACAAT AACCTGATA AATGCTTCAA TAATATTGAA
2280 AAAGGAAGAG TCCTGAGGCG GAAAGAACCA GCTGTGGAAT GTGTGTCAGT TAGGGTGTGG
2340 AAAGTCCCA GGCTCCCCAG CAGGCAGAAG TATGCAAAGC ATGCATCTCA ATTAGTCAGC
2400 AACCAGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA AGTATGCAAA GCATGCATCT
2460 CAATTAGTCA GCAACCATAG TCCCGCCCCT AACTCCGCCC ATCCCGCCCC TAACTCCGCC
2520 CAGTTCCGCC CATTCTCCGC CCCATGGCTG ACTAATTTTT TTTATTTATG CAGAGGCCGA
2580 GGCCGCTCG GCCTCTGAGC TATTCCAGAA GTAGTGAGGA GGCTTTTTTG GAGGCCTAGG
2640 CTTTTGCAAA GATCGATCAA GAGACAGGAT GAGGATCGTT TCGCATGATT GAACAAGATG
2700 GATTGCACGC AGGTTCTCCG GCCGCTTGGG TGGAGAGGCT ATTCGGCTAT GACTGGGCAC
2760 AACAGACAAT CGGCTGCTCT GATGCCGCCG TGTTCCGGCT GTCAGCGCAG GGGCGCCCGG
2820 TTCTTTTTGT CAAGACCGAC CTGTCCGGTG CCCTGAATGA ACTGCAAGAC GAGGCAGCGC
2880 GGCTATCGTG GCTGGCCACG ACGGGCGTTC CTTGCGCAGC TGTGCTCGAC GTTGTCCTG
2940 AAGCGGGAAG GGAAGGCTG CTATTGGGCG AAGTGCCGGG GCAGGATCTC CTGTCATCTC
3000 ACCTTGCTCC TGCCGAGAAA GTATCCATCA TGGCTGATGC AATGCGGCGG CTGCATACGC
3060 TTGATCCGGC TACCTGCCCA TTCGACCACC AAGCGAAACA TCGCATCGAG CGAGCACGTA
3120 CTCGGATGGA AGCCGGTCTT GTCGATCAGG ATGATCTGGA CGAAGAGCAT CAGGGGCTCG
3180 CGCCAGCCGA ACTGTTCCGC AGGCTCAAGG CGAGCATGCC CGACGGCGAG GATCTCGTCG

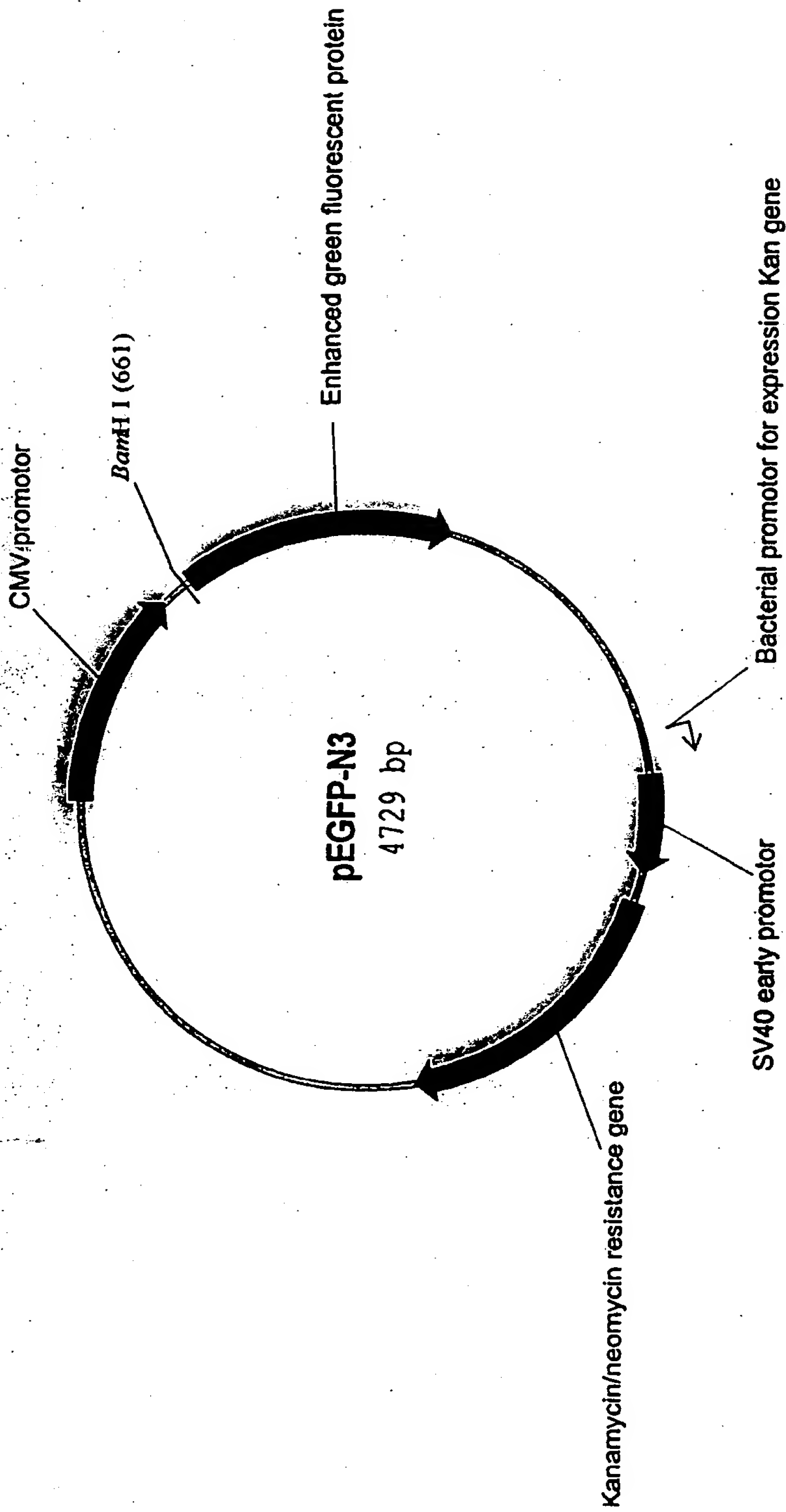
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FIG. 7 (CONTINUED)

TGACCCATGG CGATGCCTGC TTGCCGAATA TCATGGTGGA AAATGGCCGC TTTTCTGGAT
3240
TCATCGACTG TGGCCGGCTG GGTGTGGCGG ACCGCTATCA GGACATAGCG TTGGCTACCC
3300
GTGATATTGC TGAAGAGCTT GGCGGCGAAT GGGCTGACCG CTTCTCGTG CTTTACGGTA
3360
TCGCCGCTCC CGATTCGCAG CGCATCGCCT TCTATCGCCT TCTTGACGAG TTCTTCTGAG
3420
CGGGACTCTG GGGTTCGAAA TGACCGACCA AGCGACGCC AACCTGCCAT CACGAGATTT
3480
CGATTCCACC GCCGCCTTCT ATGAAAGGTT GGGCTTCGGA ATCGTTTTCC GGGACGCCGG
3540
CTGGATGATC CTCCAGCGCG GGGATCTCAT GCTGGAGTTC TTCGCCCACC CTAGGGGGAG
3600
GCTAACTGAA ACACGGAAGG AGACAATACC GGAAGGAACC CGCGCTATGA CGGCAATAAA
3660
AAGACAGAAT AAAACGCACG GTGTTGGGTC GTTTGTTTCAT AAACGCGGGG TTCGGTCCCA
3720
GGGCTGGCAC TCTGTCGATA CCCACCGAG ACCCCATTGG GGCCAATACG CCCGCGTTTC
3780
TTCCTTTTCC CCACCCACC CCCAAGTTC GGGTGAAGGC CCAGGGCTCG CAGCCAACGT
3840
CGGGGCGGCA GGCCCTGCCA TAGCCTCAGG TTAATCATAT AACTTTAGA TTGATTTAAA
3900
ACTTCATTTT TAATTTAAAA GGATCTAGGT GAAGATCCTT TTTGATAATC TCATGACCAA
3960
AATCCCTTAA CGTGAGTTTT CGTTCCTACTG AGCGTCAGAC CCCGTAGAAA AGATCAAAGG
4020
ATCTTCTTGA GATCCTTTTT TTCTGCGCGT AATCTGCTGC TTGCAAACAA AAAAACCACC
4080
GCTACCAGCG GTGGTTTGTT TGCCGGATCA AGAGCTACCA ACTCTTTTTC CGAAGGTAAC
4140
TGGCTTCAGC AGAGCGCAGA TACCAAATAC TGTCCTTCTA GTGTAGCCGT AGTTAGGCCA
4200
CCACTTCAAG AACTCTGTAG CACCGCCTAC ATACCTCGCT CTGCTAATCC TGTTACCAGT
4260
GGCTGCTGCC AGTGGCGATA AGTCGTGTCT TACCGGGTTG GACTCAAGAC GATAGTTACC
4320
GGATAAGGCG CAGCGGTCGG GCTGAACGGG GGGTTCGTGC ACACAGCCCA GCTTGGAGCG
4380
AACGACCTAC ACCGAACTGA GATACCTACA GCGTGAGCTA TGAGAAAGCG CCACGCTTCC
4440
CGAAGGGAGA AAGGCGGACA GGTATCCGGT AAGCGGCAGG GTCGGAACAG GAGAGCGCAC
4500
GAGGGAGCTT CCAGGGGGAA ACGCCTGGTA TCTTTATAGT CCTGTCGGGT TTCGCCACCT
4560
CTGACTTGAG CGTCGATTTT TGTGATGCTC GTCAGGGGGG CGGAGCCTAT GGAAAAACGC
4620
CAGCAACGCG GCCTTTTTTAC GGTTCCTGGC CTTTGTGCTG CCTTTTGCTC ACATGTTCTT
4680
TCCTGCGTTA TCCCCTGATT CTGTGGATAA CCGTATTACC GCCATGCAT
4729
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FIG. 8.



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FIG. 9.

SQ SEQUENCE 5619 BP

60 GATCCCCATG AACCGTGCTT TTAGCAGGAA GAAAGACAAA ACATGGATGC ATACACCTGA
120 AGCTTTATCA AAACATTTCA TTCCCTATAA TGCAAAGTTT CTTGGCAGTA CAGAAGTGGA
180 ACAGCCAAAA GGAACAGAAG TTGTGAGAGA TGCTGTAAGG AACTAAAGT TTGCAAGACA
240 TATCAAGAAA TCTGAAGGCC AGAAAATTCC TAAAGTGGAG TTGCAAATAT CAATTTATGG
300 AGTAAAAATT CTAGAACCCA AAACAAAGGA AGTTCAACAC AATTGCCAGC TTCATAGAAT
360 ATCTTTTTGT GCAGATGATA AAACAGACAA GAGGATATTC ACTTTCATAT GCAAAGATTC
420 TGAGTCAAAT AAACATTTGT GCTATGTATT TGACAGCGAA AAGTGTGCTG AAGAGATCAC
480 TTTAACAATT GGCCAAGCAT TTGACCTGGC ATACACGAAA TTTCTAGAAT CAGGAGGAAA
540 AGATGTTGAA ACAAGAAAAC AGATCGCAGG GTTACAAAAA AGAATCCAAG ACTTAGAAAC
600 AGAAAATATG GAACTTAAAA ATAAAGTACA AGATTTGGAA AACCAACTGA GAATAACTCA
660 AGTATCAGCA CCTCCAGCAG GCAGTATGAC ACCTAAGTCG CCCTCCACTG ACATCTTTGA
720 TATGATTCCA TTTTCTCCAA TATCACACCA GTCTTCGATG CCTACTCGCA ATGGCACACA
780 GCCACCTCCA GTACCTAGTA GATCTACTGA GATTAAACGG GACCTGTTTG GAGCAGAACC
840 TTTTGACCCA TTTAACTGTG GAGCAGCAGA TTTCCCTCCA GATATTCAAT CAAAATTAGA
900 TGAGATGCAG GAGGGGTTCA AAATGGGACT AACTCTTGAA GGCACAGTAT TTTGTCTCGA
960 CCCGTTAGAC AGTAGGTGCG TCGACGGTAC CGCGGGCCCG GGATCCATCG CCACCATGGT
1020 GAGCAAGGGC GAGGAGCTGT TCACCGGGGT GGTGCCCATC CTGGTTCGAGC TGGACGGCGA
1080 CGTAAACGGC CACAAGTTCA GCGTGTCCGG CGAGGGCGAG GGCGATGCCA CCTACGGCAA
1140 GCTGACCCTG AAGTTCATCT GCACCACCGG CAAGCTGCCC GTGCCCTGGC CCACCCTCGT
1200 GACCACCCTG ACCTACGGCG TGCAGTGCTT CAGCCGCTAC CCCGACCACA TGAAGCAGCA
1260 CGACTTCTTC AAGTCCGCCA TGCCCGAAGG CTACGTCCAG GAGCGCACCA TCTTCTTCAA
1320 GGACGACGGC AACTACAAGA CCCGCGCCGA GGTGAAGTTC GAGGGCGACA CCCTGGTGAA
1380 CCGCATCGAG CTGAAGGGCA TCGACTTCAA GGAGGACGGC AACATCCTGG GGCACAAGCT
1440 GGAGTACAAC TACAACAGCC ACAACGTCTA TATCATGGCC GACAAGCAGA AGAACGGCAT
1500 CAAGGTGAAC TTCAAGATCC GCCACAACAT CGAGGACGGC AGCGTGCAGC TCGCCGACCA

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FIG. 9. (CONTINUED)

CTACCAGCAG AACACCCCCA TCGGCGACGG CCCCCTGCTG CTGCCCCGACA ACCACTACCT
1560
GAGCACCCAG TCCGCCCTGA GCAAAGACCC CAACGAGAAG CGCGATCACA TGGTCCTGCT
1620
GGAGTTCGTG ACCGCCGCCG GGATCACTCT CGGCATGGAC GAGCTGTACA AGTAAAGCGG
1680
CCGCGACTCT AGATCATAAT CAGCCATACC ACATTTGTAG AGGTTTTACT TGCTTTAAAA
1740
AACCTCCCAC ACCTCCCCCT GAACCTGAAA CATAAAATGA ATGCAATTGT TGTGTTAAC
1800
TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTCACAAAT
1860
AAAGCATTTT TTCACTGCA TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAA
1920
GGCGTAAATT GTAAGCGTTA ATATTTTGT AAAATTCGCG TTAAATTTT GTTAAATCAG
1980
CTCATTTTTT AACCAATAGG CCGAAATCGG CAAAATCCCT TATAATCAA AAGAATAGAC
2040
CGAGATAGGG TTGAGTGTTG TTCCAGTTTG GAACAAGAGT CCACTATTAA AGAACGTGGA
2100
CTCCAACGTC AAAGGGCGAA AAACCGTCTA TCAGGGCGAT GGCCCACTAC GTGAACCATC
2160
ACCCTAATCA AGTTTTTTGG GGTCGAGGTG CCGTAAAGCA CTAAATCGGA ACCCTAAAGG
2220
GAGCCCCCGA TTTAGAGCTT GACGGGGAAA GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA
2280
GAAAGCGAAA GGAGCGGGCG CTAGGGCGCT GGCAAGTGTA GCGGTCACGC TCGCGGTAAC
2340
CACCACACCC GCCGCGCTTA ATGCGCCGCT ACAGGGCGCG TCAGGTGGCA CTTTTCGGGG
2400
AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT
2460
CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA GTCCTGAGGC
2520
GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA
2580
GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
2640
CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA
2700
GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAATCCGC CCAGTTCCGC CCATTCTCCG
2760
CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG
2820
CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG GCTTTTGCAA AGATCGATCA
2880
AGAGACAGGA TGAGGATCGT TTCGCATGAT TGAACAAGAT GGATTGCACG CAGGTTCTCC
2940
GGCCGCTTGG GTGGAGAGGC TATTCGGCTA TGAAGGGCA CAACAGACAA TCGGCTGCTC
3000
TGATGCCGCC GTGTTCCGGC TGTGAGCGCA GGGGCGCCCG GTTCTTTTTG TCAAGACCGA
3060
CCTGTCCGGT GCCCTGAATG AACTGCAAGA CGAGGCAGCG CGGCTATCGT GGCTGGCCAC
3120
GACGGGCGTT CCTTGCGCAG CTGTGCTCGA CGTTGTCACT GAAGCGGGAA GGGACTGGCT
3180

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FIG. 9. (CONTINUED)

GCTATTGGGC GAAGTGCCGG GGCAGGATCT CCTGTCATCT CACCTTGCTC CTGCCGAGAA
3240
AGTATCCATC ATGGCTGATG CAATGCGGCG GCTGCATACG CTTGATCCGG CTACCTGCCC
3300
ATTCGACCAC CAAGCGAAAC ATCGCATCGA GCGAGCACGT ACTCGGATGG AAGCCGGTCT
3360
TGTCGATCAG GATGATCTGG ACGAAGAGCA TCAGGGGCTC GCGCCAGCCG AACTGTTTCG
3420
CAGGCTCAAG GCGAGCATGC CCGACGGCGA GGATCTCGTC GTGACCCATG GCGATGCCTG
3480
CTTGCCGAAT ATCATGGTGG AAAATGGCCG CTTTTCTGGA TTCATCGACT GTGGCCGGCT
3540
GGGTGTGGCG GACCGCTATC AGGACATAGC GTTGGCTACC CGTGATATTG CTGAAGAGCT
3600
TGGCGGCGAA TGGGCTGACC GCTTCCTCGT GCTTTACGGT ATCGCCGCTC CCGATTCGCA
3660
GCGCATCGCC TTCTATCGCC TTCTTGACGA GTTCTTCTGA GCGGGACTCT GGGGTTCGAA
3720
ATGACCGACC AAGCGACGCC CAACCTGCCA TCACGAGATT TCGATTCCAC CGCCGCCTTC
3780
TATGAAAGGT TGGGCTTCGG AATCGTTTTT CGGGACGCCG GCTGGATGAT CCTCCAGCGC
3840
GGGGATCTCA TGCTGGAGTT CTTCGCCCAC CCTAGGGGGA GGCTAACTGA AACACGGAAG
3900
GAGACAATAC CGGAAGGAAC CCGCGCTATG ACGGCAATAA AAAGACAGAA TAAACGCAC
3960
GGTGTGGGT CGTTTGTTCA TAAACGCGGG GTTCGGTCCC AGGGCTGGCA CTCTGTCGAT
4020
ACCCACCGA GACCCCATTG GGGCCAATAC GCCCGCGTTT CTCCTTTTC CCCACCCAC
4080
CCCCAAGTT CGGGTGAAGG CCCAGGGCTC GCAGCCAACG TCGGGGCGGC AGGCCCTGCC
4140
ATAGCCTCAG GTTACTCATA TATACTTTAG ATTGATTAA AACTTCATTT TTAATTTAAA
4200
AGGATCTAGG TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT
4260
TCGTTCCACT GAGCGTCAGA CCCCCTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT
4320
TTTCTGCGCG TAATCTGCTG CTTGCAAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT
4380
TTGCCGGATC AAGAGCTACC AACTCTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG
4440
ATACCAAATA CTGTCCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA
4500
GCACCGCCTA CATACTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT
4560
AAGTCGTGTC TTACCGGGTT GGA CTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCTG
4620
GGCTGAACGG GGGGTTCTGT CACACAGCCC AGCTTGGAGC GAACGACCTA CACCGAACTG
4680
AGATACCTAC AGCGTGAGCT ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC
4740
AGGTATCCGG TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA
4800
AACGCCTGGT ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA GCGTCGATTT
4860

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FIG. 9. (CONTINUED)

4920 TTGTGATGCT CGTCAGGGGG GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA
4980 CGGTTCTGCT CTTTTGCTG GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT
5040 TCTGTGGATA ACCGTATTAC CGCCATGCAT TAGTTATTAA TAGTAATCAA TTACGGGGTC
5100 ATTAGTTCAT AGCCCATATA TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC
5160 TGGCTGACCG CCCAACGACC CCCGCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
5220 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT AAAGTGGCCA
5280 CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC CCTATTGACG TCAATGACGG
5340 TAAATGGCCC GCCTGGCATT ATGCCCAGTA CATGACCTTA TGGGACTTTC CTACTTGGCA
5400 GTACATCTAC GTATTAGTCA TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA
5460 TGGGCGTGGA TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
5520 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA ACAACTCCGC
5580 CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG GTCTATATAA GCAGAGCTGG
5619 TTTAGTGAAC CGTCAGATCC GCTAGCGCTA CCGGACTCA
//

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FIG. 10

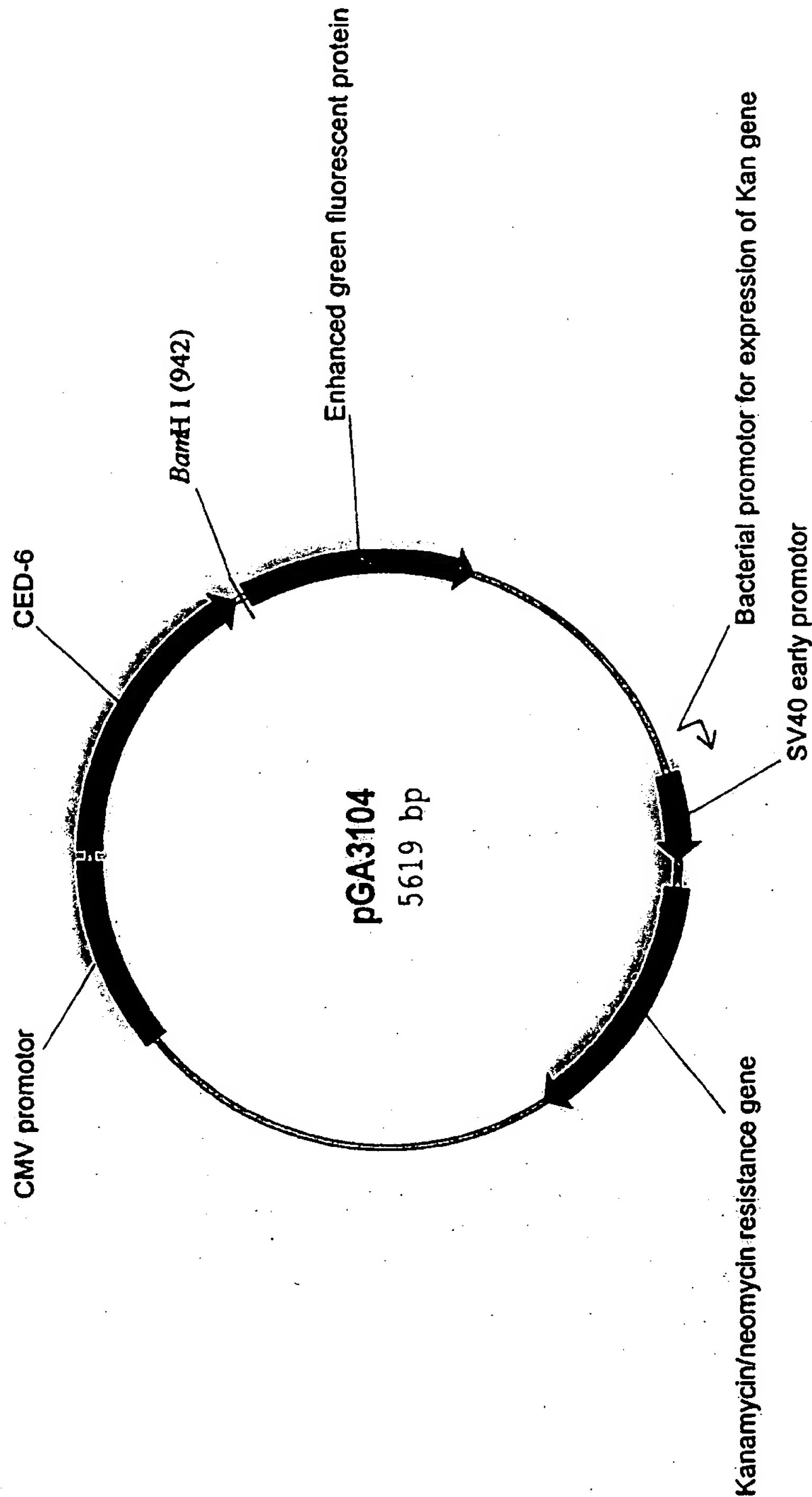


FIG. 11. 23/56

ID pcDNA3.1/His/LacZ

circular DNA; 8578 BP

SQ	SEQUENCE	8578 BP;	
	GACGGATCGG	GAGATCTCCC	GATCCCCTAT
	CCGCATAGTT	AAGCCAGTAT	CTGCTCCCTG
	CGAGCAAAT	TTAAGCTACA	ACAAGGCAAG
	TTAGGGTTAG	GCGTTTTCG	CTGCTTCGCG
	GATTATTGAC	TAGTTATTAA	TAGTAATCAA
	TGGAGTTCCG	CGTTACATAA	CTTACGGTAA
	CCCGCGCATT	GACGTCAATA	ATGACGTATG
	ATTGACGTCA	ATGGGTGGAC	TATTTACGGT
	ATCATATGCC	AAGTACGCCC	CCTATTGACG
	ATGCCAGTA	CATGACCTTA	TGGGACTTTC
	TCGCTATTAC	CATGGTGATG	CGGTTTTGGC
	ACTCACGGGG	ATTTCCAAGT	CTCCACCCCA
	AAAATCAACG	GGACTTTCCA	AAATGTCGTA
	GTAGGCGTGT	ACGGTGGGAG	GTCTATATAA
	CTGCTTACTG	GCTTATCGAA	ATTAATACGA
	GTTTAAACTT	AAGCTTACCA	TGGGGGGTTC
	CATGACTGGT	GGACAGCAAA	TGGGTCGGGA
	GGATCAGCTT	GGAGTTGATC	CCGTCGTTTT
	TACCCAACCTT	AATCGCCTTG	CAGCACATCC
	GGCCCGCACC	GATCGCCCTT	CCCAACAGTT
	CTGGTTTCCG	GCACCAGAAG	CGGTGCCGGA
	CGATACTGTC	GTCGTCCCCT	CAAACCTGGCA
	CAACGTAACC	TATCCCATT	CGGTCAATCC
	TTGTTACTCG	CTCACATTTA	ATGTTGATGA
	TATTTTGTAT	GGCGTTAACT	CGGCGTTTCA
	CGGCCAGGAC	AGTCGTTTGC	CGTCTGAATT
	AAACCGCCTC	GCGGTGATGG	TGCTGCGTTG
	TATGTGGCGG	ATGAGCGGCA	TTTTCCGTGA
	AATCAGCGAT	TTCCATGTTG	CCACTCGCTT
	GGCTGAAGTT	CAGATGTGCG	GCGAGTTGCG
	GCAGGGTGAA	ACGCAGGTCG	CCAGCGGCAC
	GCGTGGTGGT	TATGCCGATC	GCGTCACACT
	GAGCGCCGAA	ATCCCGAATC	TCTATCGTGC
	GCTGATTGAA	GCAGAAAGCT	GCGATGTCGG
	GCTGCTGCTG	AACGGCAAGC	CGTTGCTGAT
	TCTGCATGGT	CAGGTCATGG	ATGAGCAGAC
	GAACAACCTT	AACGCCGTGC	GCTGTTGCGA
	GTGCGACCGC	TACGGCCTGT	ATGTGGTGGA
	GCCAATGAAT	CGTCTGACCG	ATGATCCGCG
	GCGAATGGTG	CAGCGCGATC	GTAATCACCC
	ATCAGGCCAC	GGCGCTAATC	ACGACGCGCT
	CCGCCCGGTG	CAGTATGAG	GCGGCGGAGC
	GATGTACGCG	CGCGTGGATG	AAGACCAGCC
	AAAATGGCTT	TCGCTACCTG	GAGAGACGCG
	GATGGGTAAAC	AGTCTTGGCG	GTTTCGCTAA
	TTTACAGGGC	GGCTTCGTCT	GGGACTGGGT
	CGGCAACCCG	TGGTCGGCTT	ACGGCGGTGA
	CTGTATGAAC	GGTCTGGTCT	TTGCCGACCG
	ACACCAGCAG	CAGTTTTTCC	AGTTCCGTTT
	ATACCTGTTC	CGTCATAGCG	ATAACGAGCT
	GCCGCTGGCA	AGCGGTGAG	TGCCTCTGGA
	ACTGCCTGAA	CTACCGCAGC	CGGAGAGCGC
	GCAACCGAAC	GCGACCGCAT	GGTCAGAAGC
	TCTGGCGGAA	AACCTCACTG	TGACGCTCCC
			CGCCGCGTCC
			CACGCCATCC
			CGCATCTGAC

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FIG. 11. (CONTINUED)

CACCAGCGAA	ATGGATTTTT	GCATCGAGCT	GGGTAATAAG	CGTTGGCAAT	TTAACCGCCA	3300
GTCAGGCTTT	CTTTCACAGA	TGTGGATTGG	CGATAAAAAA	CAACTGCTGA	CGCCGCTGCG	3360
CGATCAGTTC	ACCCGTGCAC	CGCTGGATAA	CGACATTGGC	GTAAGTGAAG	CGACCCGCAT	3420
TGACCCTAAC	GCCTGGGTCG	AACGCTGGAA	GGCGGCGGGC	CATTACCAGG	CCGAAGCAGC	3480
GTTGTTGCAG	TGCACGGCAG	ATACACTTGC	TGATGCGGTG	CTGATTACGA	CCGCTCACGC	3540
GTGGCAGCAT	CAGGGGAAAA	CCTTATTTAT	CAGCCGGAAA	ACCTACCGGA	TTGATGGTAG	3600
TGGTCAAATG	GCGATTACCG	TTGATGTTGA	AGTGGCGAGC	GATACACCGC	ATCCGGCGCG	3660
GATTGGCCTG	AACTGCCAGC	TGGCGCAGGT	AGCAGAGCGG	GTAAACTGGC	TCGGATTAGG	3720
GCCGCAAGAA	AACTATCCCG	ACCGCCTTAC	TGCCGCCTGT	TTTGACCGCT	GGGATCTGCC	3780
ATTGTCAGAC	ATGTATACCC	CGTACGTCTT	CCCGAGCGAA	AACGGTCTGC	GCTGCGGGAC	3840
GCGCGAATTG	AATTATGGCC	CACACCAGTG	GCGCGGCGAC	TTCCAGTTCA	ACATCAGCCG	3900
CTACAGTCAA	CAGCAACTGA	TGGAAACCAG	CCATCGCCAT	CTGCTGCACG	CGGAAGAAGG	3960
CACATGGCTG	AATATCGACG	GTTTCCATAT	GGGGATTGGT	GGCGACGACT	CCTGGAGCCC	4020
GTCAGTATCG	GCGGAGTTCC	AGCTGAGCGC	CGGTCGCTAC	CATTACCAGT	TGGTCTGGTG	4080
TCAAAAATAA	TAAAGCCGAA	TTCTGCAGAT	ATCCAGCACA	GTGGCGGCCG	CTCGAGTCTA	4140
GAGGGCCCGT	TTAAACCCGC	TGATCAGCCT	CGACTGTGCC	TTCTAGTTGC	CAGCCATCTG	4200
TTGTTTGCCC	CTCCCCCGTG	CCTTCCTTGA	CCCTGGAAGG	TGCCACTCCC	ACTGTCCTTT	4260
CCTAATAAAA	TGAGGAATT	GCATCGCATT	GTCTGAGTAG	GTGTCATTCT	ATTCTGGGGG	4320
GTGGGGTGGG	GCAGGACAGC	AAGGGGGAGG	ATTGGGAAGA	CAATAGCAGG	CATGCTGGGG	4380
ATGCGGTGGG	CTCTATGGCT	TCTGAGGCGG	AAAGAACCAG	CTGGGGCTCT	AGGGGGTATC	4440
CCCACGCGCC	CTGTAGCGGC	GCATTAAGCG	CGGCGGGTGT	GGTGGTTACG	CGCAGCGTGA	4500
CCGCTACACT	TGCCAGCGCC	CTAGCGCCCC	CTCCTTTCGC	TTTCTTCCCT	TCCTTTCTCG	4560
CCACGTTTCG	CGGCTTTCCC	CGTCAAGCTC	TAAATCGGGG	CATCCCTTTA	GGGTTCGAT	4620
TTAGTGCTTT	ACGGCACCTC	GACCCCAAAA	AACTTGATTA	GGGTGATGGT	TCACGTAGTG	4680
GGCCATCGCC	CTGATAGACG	GTTTTTCGCC	CTTTGACGTT	GGAGTCCACG	TTCTTTAATA	4740
GTGGACTCTT	GTTCCAAACT	GGAACAACAC	TCAACCCTAT	CTCGGTCTAT	TCTTTTGATT	4800
TATAAGGGAT	TTTGGGGATT	TCGGCCTATT	GGTTAAAAAA	TGAGCTGATT	TAACAAAAAT	4860
TTAACGCGAA	TTAATTCTGT	GGAATGTGTG	TCAGTTAGGG	TGTGGAAAGT	CCCCAGGCTC	4920
CCCAGGCAGG	CAGAAGTATG	CAAAGCATGC	ATCTCAATTA	GTCAGCAACC	AGGTGTGGAA	4980
AGTCCCCAGG	CTCCCCAGCA	GGCAGAAGTA	TGCAAAGCAT	GCATCTCAAT	TAGTCAGCAA	5040
CCATAGTCCC	GCCCCTAACT	CCGCCCATCC	CGCCCCTAAC	TCCGCCCAGT	TCCGCCCAT	5100
CTCCGCCCCA	TGGCTGACTA	ATTTTTTTTA	TTTATGCAGA	GGCCGAGGCC	GCCTCTGCCT	5160
CTGAGCTATT	CCAGAAGTAG	TGAGGAGGCT	TTTTTGGAGG	CCTAGGCTTT	TGCAAAAAGC	5220
TCCCGGGAGC	TTGTATATCC	ATTTTCGGAT	CTGATCAAGA	GACAGGATGA	GGATCGTTTC	5280
GCATGATTGA	ACAAGATGGA	TTGCACGCAG	GTTCTCCGGC	CGCTTGGGTG	GAGAGGCTAT	5340
TCGGCTATGA	CTGGGCACAA	CAGACAATCG	GCTGCTCTGA	TGCCGCCGTG	TTCCGGCTGT	5400
CAGCGCAGGG	GCGCCCGGTT	CTTTTTGTCA	AGACCGACCT	GTCCGGTGCC	CTGAATGAAC	5460
TGCAGGACGA	GGCAGCGCGG	CTATCGTGCG	TGGCCACGAC	GGGCGTTCCT	TGCGCAGCTG	5520
TGCTCGACGT	TGTCACTGAA	GCGGGAAGGG	ACTGGCTGCT	ATTGGGCGAA	GTGCCGGGGC	5580
AGGATCTCCT	GTCATCTCAC	CTTGCTCCTG	CCGAGAAAGT	ATCCATCATG	GCTGATGCAA	5640
TGCGGCGGCT	GCATACGCTT	GATCCGGCTA	CCTGCCCAT	CGACCACCAA	GCGAAACATC	5700
GCATCGAGCG	AGCACGTACT	CGGATGGAAG	CCGGTCTTGT	CGATCAGGAT	GATCTGGACG	5760
AAGAGCATCA	GGGGCTCGCG	CCAGCCGAAC	TGTTCCGCCAG	GCTCAAGGCG	CGCATGCCCG	5820
ACGGCGAGGA	TCTCGTCGTG	ACCCATGGCG	ATGCCTGCTT	GCCGAATATC	ATGGTGGAAG	5880
ATGGCCGCTT	TTCTGGATTG	ATCGACTGTG	GCCGGCTGGG	TGTGGCGGAC	CGCTATCAGG	5940
ACATAGCGTT	GGCTACCCGT	GATATTGCTG	AAGAGCTTGG	CGGCGAATGG	GCTGACCGCT	6000
TCCTCGTGCT	TTACGGTATC	GCCGCTCCCC	ATTGCGAGCG	CATCGCCTTC	TATCGCCTTC	6060
TTGACGAGTT	CTTCTGAGCG	GGACTCTGGG	GTTGGAATG	ACCGACCAAG	CGACGCCCAA	6120
CCTGCCATCA	CGAGATTTCG	ATTCCACCGC	CGCCTTCTAT	GAAAGGTTGG	GCTTCGGAAT	6180
CGTTTTCCGG	GACGCCCGCT	GGATGATCCT	CCAGCGCGGG	GATCTCATGC	TGGAGTTCTT	6240
CGCCACCCC	AACTTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	6300
AAATTTTACA	AATAAAGCAT	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	6360
CAATGTATCT	TATCATGTCT	GTATACCGTC	GACCTCTAGC	TAGAGCTTGG	CGTAATCATG	6420
GTCATAGCTG	TTTCCTGTGT	GAAATTGTTA	TCCGCTCACA	ATTCCACACA	ACATACGAGC	6480
CGGAAGCATA	AAGTGTAAG	CCTGGGGTGC	CTAATGAGTG	AGCTAACTCA	CATTAATTGC	6540
GTTGCGCTCA	CTGCCCGCTT	TCCAGTCGGG	AAACCTGTGC	TGCCAGCTGC	ATTAATGAAT	6600
CGGCCAACGC	GCGGGGAGAG	GCGGTTTGCG	TATTGGGCGC	TCTTCCGCTT	CCTCGCTCAC	6660

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FIG. 11. (CONTINUED)

TGACTCGCTG	CGCTCGGTCG	TTCGGCTGCG	GCGAGCGGTA	TCAGCTCACT	CAAAGGCGGT	6720
AATACGGTTA	TCCACAGAAT	CAGGGGATAA	CGCAGGAAAG	AACATGTGAG	CAAAAGGCCA	6780
GCAAAGGCC	AGGAACCGTA	AAAAGGCCGC	GTTGCTGGCG	TTTTTCCATA	GGCTCCGCCC	6840
CCCTGACGAG	CATCACAAA	ATCGACGCTC	AAGTCAGAGG	TGGCGAAACC	CGACAGGACT	6900
ATAAGATAC	CAGGCGTTTC	CCCCTGGAAG	CTCCCTCGTG	CGCTCTCCTG	TTCCGACCCT	6960
GCCGCTTACC	GGATACCTGT	CCGCCTTTCT	CCCTTCGGGA	AGCGTGCGC	TTTCTCAATG	7020
CTCAGCTGT	AGGTATCTCA	GTTGCGTGTA	GGTCGTTTCG	TCCAAGCTGG	GCTGTGTGCA	7080
CGAACCCCC	GTTCAGCCCG	ACCGCTGCGC	CTTATCCGGT	AACTATCGTC	TTGAGTCCAA	7140
CCCGGTAAGA	CACGACTTAT	CGCCACTGGC	AGCAGCCACT	GGTAACAGGA	TTAGCAGAGC	7200
GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	CCTAACTACG	GCTACACTAG	7260
AAGGACAGTA	TTTGGTATCT	GCGCTCTGCT	GAAGCCAGTT	ACCTTCGGAA	AAAGAGTTGG	7320
TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	GGTTTTTTTG	TTTGCAAGCA	7380
GCAGATTACG	CGCAGAAAA	AAGGATCTCA	AGAAGATCCT	TTGATCTTTT	CTACGGGGTC	7440
TGACGCTCAG	TGGAACGAAA	ACTCACGTTA	AGGGATTTTG	GTCATGAGAT	TATCAAAAAG	7500
GATCTTCACC	TAGATCCTTT	TAAATTAATA	ATGAAGTTTT	AAATCAATCT	AAAGTATATA	7560
TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	GAGGCACCTA	TCTCAGCGAT	7620
CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC	GTGTAGATAA	CTACGATACG	7680
GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC	AATGATACCG	CGAGACCCAC	GCTCACCAGC	7740
TCCAGATTTA	TCAGCAATAA	ACCAGCCAGC	CGGAAGGGCC	GAGCGCAGAA	GTGGTCCTGC	7800
AACTTTATCC	GCCTCCATCC	AGTCTATTAA	TTGTTGCCGG	GAAGCTAGAG	TAAGTAGTTC	7860
GCCAGTTAAT	AGTTTGCGCA	ACGTTGTTGC	CATTGCTACA	GGCATCGTGG	TGTCACGCTC	7920
GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA	TCAAGGCGAG	TTACATGATC	7980
CCCCATGTTG	TGCAAAAAG	CGGTTAGCTC	CTTCGGTCCT	CCGATCGTTG	TCAGAAGTAA	8040
GTTGGCCGCA	GTGTTATCAC	TCATGGTTAT	GGCAGCACTG	CATAATTCTC	TTACTGTCAT	8100
GCCATCCGTA	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	ACCAAGTCAT	TCTGAGAATA	8160
GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAATA	CGGGATAATA	CCGCGCCACA	8220
TAGCAGAACT	TTAAAAGTGC	TCATCATTGG	AAAACGTTCT	TCGGGGCGAA	AACTCTCAAG	8280
GATCTTACCG	CTGTTGAGAT	CCAGTTCGAT	GTAACCCACT	CGTGACCCA	ACTGATCTTC	8340
AGCATCTTTT	ACTTTCACCA	GCGTTTCTGG	GTGAGCAAAA	ACAGGAAGGC	AAAATGCCGC	8400
AAAAAAGGGA	ATAAGGGCGA	CACGGAAATG	TTGAATACTC	ATACTCTTCC	TTTTTCAATA	8460
TTATTGAAGC	ATTTATCAGG	GTTATTGTCT	CATGAGCGGA	TACATATTTG	AATGTATTTA	8520
GAAAAATAAA	CAAAATAGGG	TTCCGCGCAC	ATTTCCCCGA	AAAGTGCCAC	CTGACGTC	8578

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FIG. 12.

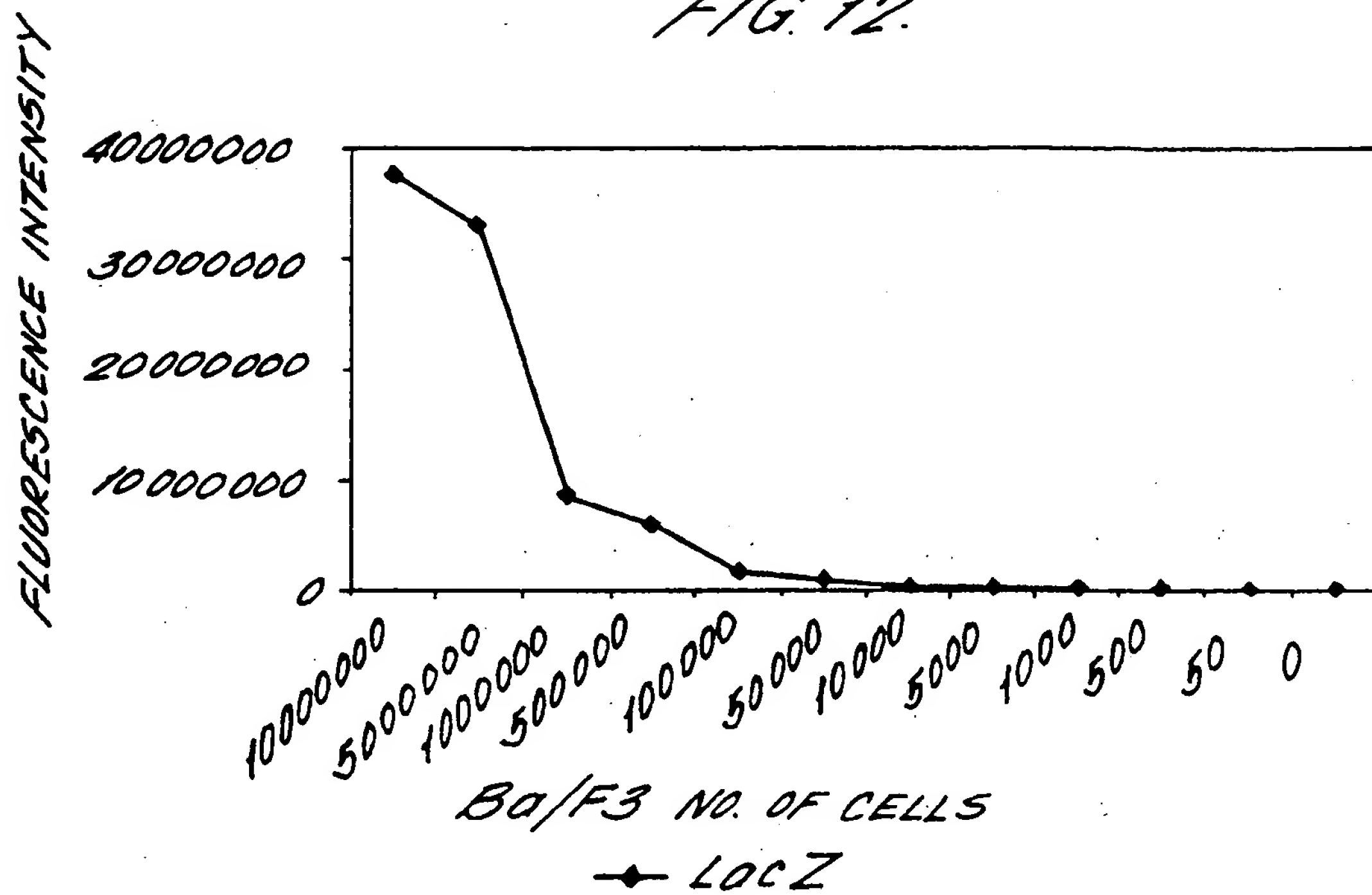
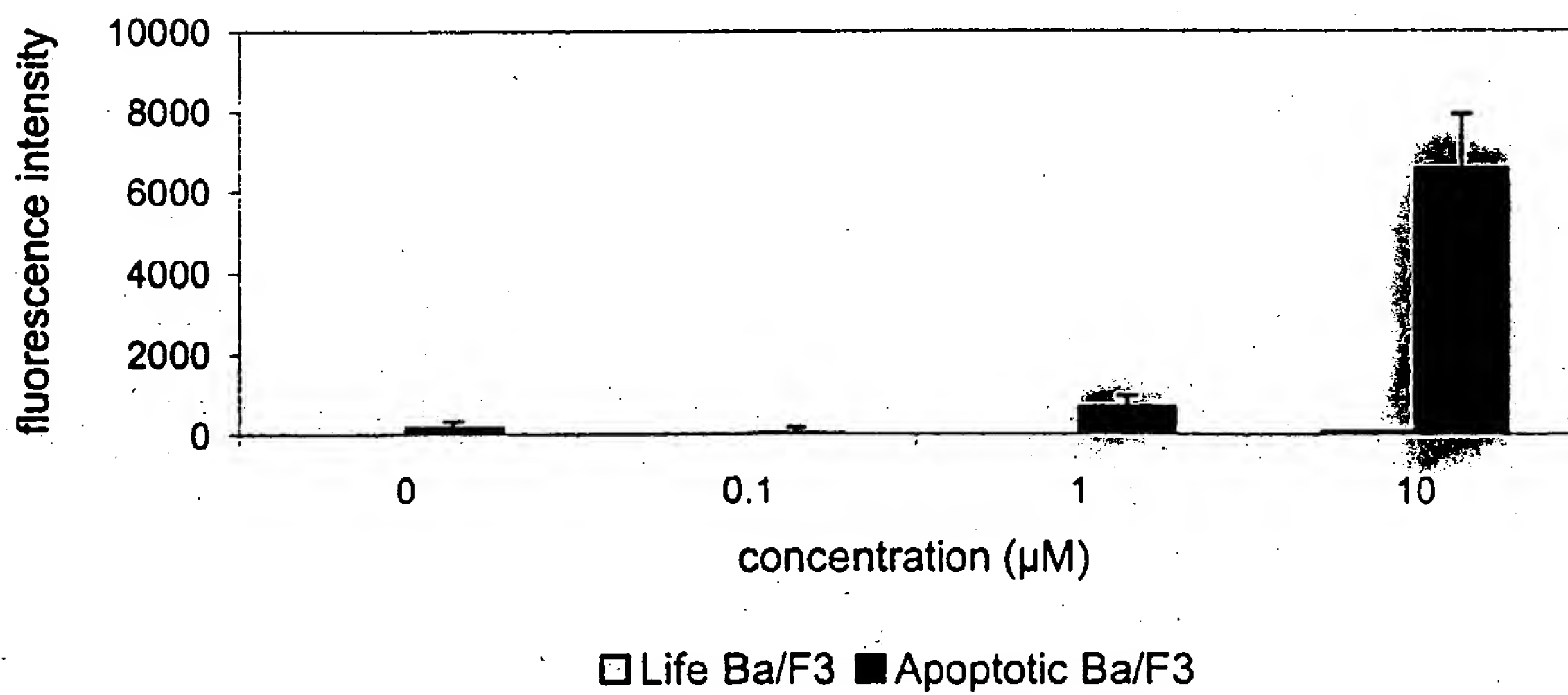


FIG. 13.



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FIG. 14.

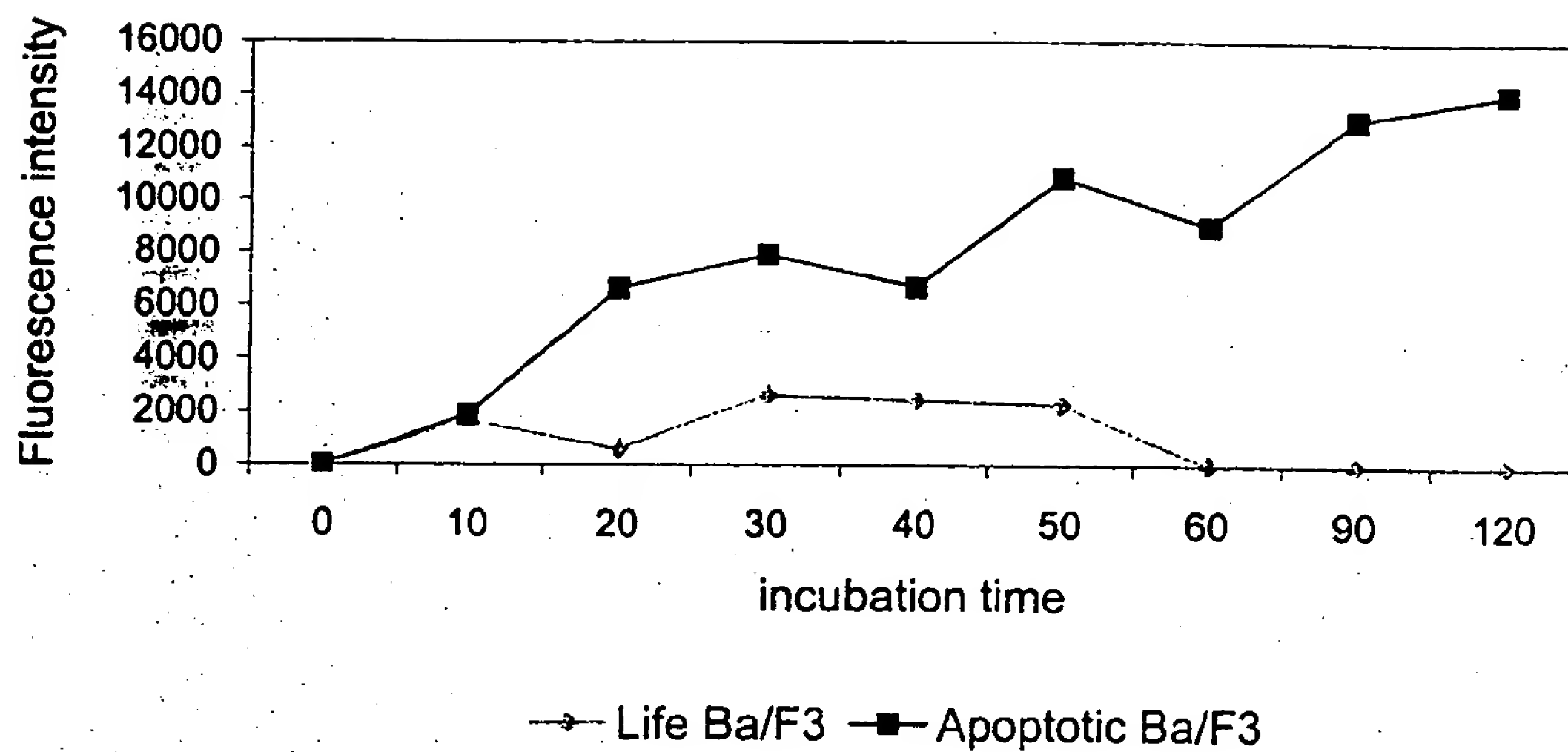
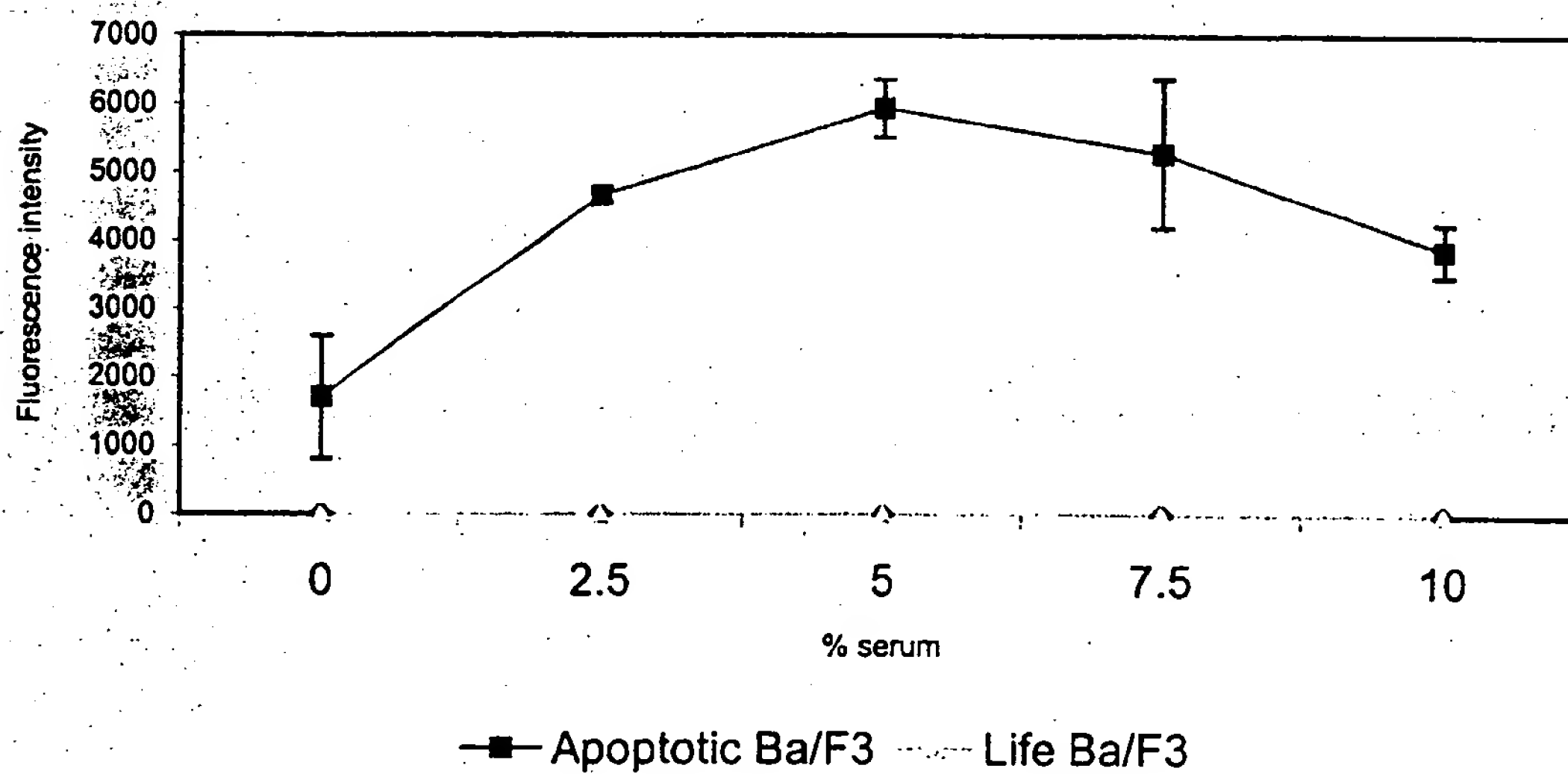


FIG. 15.



*28/56**FIG. 16.*

mnrafsrkkdktwmhtpealskhfipynakflgsteveqpkgtevvrdavrklkfarhikksegqkipk
velqisiygvkilepktkevqhncqlhrisfcaddktdkriftfickdsesnhlcyvfdsekcaeeitligq
afdlaytkflesggkdvetrkqiaglkriqdletenmelknkvqdlenqlritqvsappagsmtpkspst
difdmipfspishqssmptnngtqpppvpsrsteikrdlfgaepfdpfncgaadfppdiqskldemqegf
kmgltlegtvfclpldsrc

FIG. 17

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ID pGA1028 circular DNA; 5021 BP
DE hCed-6cds in pBAD HisA
CC <http://www.informaxinc.com/>
CC pGA1028 in Top 10:
CC VNTAUTHORNAME|Nina cromheecke|
FT CDS 8..919
FT /vntifkey="4"
FT /label=hsCED-6
FT CDS 4918..4935
FT /vntifkey="4"
FT /label=HIS-tag
FT misc_feature 4975..4998
FT /vntifkey="21"
FT /label=anti\Xpress\epitope
FT misc_feature 4954..4998
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FT /vntifkey="4"
FT /label=AraC\ORF
SQ SEQUENCE 5021 BP;

GATCCCCATG	AACCGTGCTT	TTAGCAGGAA	GAAAGACAAA	ACATGGATGC	ATACACCTGA	60
AGCTTTATCA	AAACATTTCA	TTCCCTATAA	TGCAAAGTTT	CTTGGCAGTA	CAGAAGTGGA	120
ACAGCCAAA	GGAACAGAG	TTGTGAGAGA	TGCTGTAAGG	AAACTAAAGT	TTGCAAGACA	180
TATCAAGAAA	TCTGAAGGCC	AGAAAATTCC	TAAAGTGGAG	TTGCAAATAT	CAATTTATGG	240
AGTAAAAATT	CTAGAACCCA	AAACAAAGGA	AGTTCAACAC	AATTGCCAGC	TTCATAGAAT	300
ATCTTTTTGT	GCAGATGATA	AAACTGACAA	GAGGATATTC	ACTTTCATAT	GCAAAGATTC	360
TGAGTCAAA	AAACATTTGT	GCTATGTATT	TGACAGCGAA	AAGTGTGCTG	AAGAGATCAC	420
TTTAACAATT	GGCCAAGCAT	TTGACCTGGC	ATACACGAAA	TTTCTAGAAT	CAGGAGGAAA	480
AGATGTTGAA	ACAAGAAAC	AGATCGCAGG	GTTACAAAA	AGAATCCAAG	ACTTAGAAAC	540
AGAAAATATG	GAACCTAAAA	ATAAAGTACA	AGATTTGGAA	AACCAACTGA	GAATAACTCA	600
AGTATCAGCA	CCTCCAGCAG	GCAGTATGAC	ACCTAAGTCG	CCCTCCACTG	ACATCTTTGA	660
TATGATTCCA	TTTTCTCCAA	TATCACACCA	GTCTTCGATG	CCTACTCGCA	ATGGCACACA	720
GCCACCTCCA	GTACCTAGTA	GATCTACTGA	GATTAAACGG	GACCTGTTTG	GAGCAGAACC	780
TTTTGACCCA	TTTAACTGTG	GAGCAGCAGA	TTCCCTCCA	GATATTCAAT	CAAAATTAGA	840
TGAGATGCAG	GAGGGGTTC	AAATGGGACT	AACTCTTGAA	GGCACAGTAT	TTTGTCTCGA	900
CCCGTTAGAC	AGTAGGTGCT	GAGTCGACGG	TACCATATGG	GAATTGGAAG	CTTGGCTGTT	960
TTGGCGGATG	AGAGAAGATT	TTCAGCCTGA	TACAGATTAA	ATCAGAACGC	AGAAGCGGTC	1020
TGATAAAACA	GAATTTGCCT	GGCGGCAGTA	GCGCGGTGGT	CCCACCTGAC	CCCATGCCGA	1080
ACTCAGAAGT	GAAACGCCGT	AGCGCCGATG	GTAAGTGGG	GTCTCCCAT	GCGAGAGTAG	1140
GGAACCTGCC	GGCATCAAAT	AAAACGAAAG	GCTCAGTCGA	AAGACTGGGC	CTTTCGTTTT	1200
ATCTGTTGTT	TGTCGGTGAA	CGCTCTCCTG	AGTAGGACAA	ATCCGCCGGG	AGCGGATTTG	1260
AACGTTGCGA	AGCAACGGCC	CGGAGGGTGG	CGGGCAGGAC	GCCCGCCATA	AACTGCCAGG	1320
CATCAAATTA	AGCAGAAAGC	CATCCTGACG	GATGGCCTTT	TTGCGTTTCT	ACAAACTCTT	1380
TTTGTATTAT	TTTCTAAATA	CATTCAAATA	TGTATCCGCT	CATGAGACAA	TAACCCTGAT	1440
AAATGCTTCA	ATAATATTGA	AAAAGGAAGA	GTATGAGTAT	TCAACATTTT	CGTGTGCCCC	1500
TTATTCCCTT	TTTTGCGGCA	TTTTGCCTTC	CTGTTTTTGC	TCACCCAGAA	ACGCTGGTGA	1560
AAGTAAAAGA	TGCTGAAGAT	CAGTTGGGTG	CACGAGTGGG	TTACATCGAA	CTGGATCTCA	1620
ACAGCGGTAA	GATCCTTGAG	AGTTTTCGCC	CCGAAGAACG	TTTTCCAATG	ATGAGCACTT	1680
TTAAAGTTCT	GCTATGTGGC	GCGGTATTAT	CCCGTGTGTA	CGCCGGGCAA	GAGCAACTCG	1740
GTCGCCGCAT	ACACTATTCT	CAGAATGACT	TGGTTGAGTA	CTCACCAGTC	ACAGAAAAGC	1800

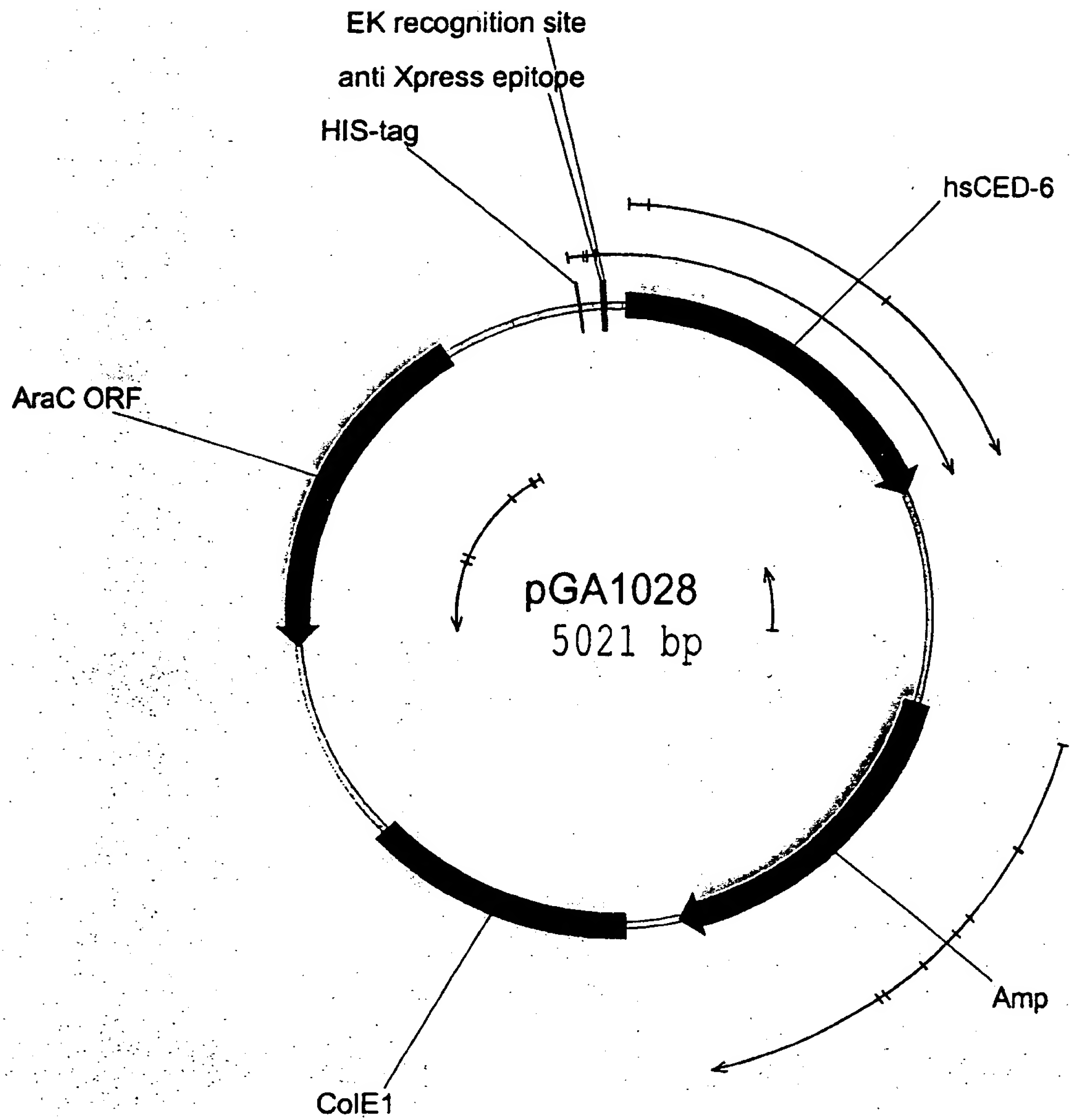
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FIG. 17 (CONTINUED)

ATCTTACGGA	TGGCATGACA	GTAAGAGAAT	TATGCAGTGC	TGCCATAACC	ATGAGTGATA	1860
ACACTGCGGC	CAACTTACTT	CTGACAACGA	TCGGAGGACC	GAAGGAGCTA	ACCGCTTTTT	1920
TGCACAACAT	GGGGGATCAT	GTAACTCGCC	TTGATCGTTG	GGAACCGGAG	CTGAATGAAG	1980
CCATACCAAA	CGACGAGCGT	GACACCACGA	TGCTGTAGC	AATGGCAACA	ACGTTGCGCA	2040
AACTATTAAC	TGGCGAACTA	CTTACTCTAG	CTTCCCGGCA	ACAATTAATA	GACTGGATGG	2100
AGGCGGATAA	AGTTGCAGGA	CCACTTCTGC	GCTCGGCCCT	TCCGGCTGGC	TGGTTTATTG	2160
CTGATAAATC	TGGAGCCGGT	GAGCGTGGGT	CTCGCGGTAT	CATTGCAGCA	CTGGGGCCAG	2220
ATGGTAAGCC	CTCCCGTATC	GTAGTTATCT	ACACGACGGG	GAGTCAGGCA	ACTATGGATG	2280
AACGAAATAG	ACAGATCGCT	GAGATAGGTG	CCTCACTGAT	TAAGCATTGG	TAAGTGTGAG	2340
ACCAAGTTTA	CTCATATATA	CTTTAGATTG	ATTTAAAACT	TCATTTTAA	TTTAAAGGA	2400
TCTAGGTGAA	GATCCTTTTT	GATAATCTCA	TGACCAAAAT	CCCTTAACGT	GAGTTTTCGT	2460
TCCACTGAGC	GTCAGACCCC	GTAGAAAAGA	TCAAAGGATC	TTCTTGAGAT	CCTTTTTTTC	2520
TGCGCGTAAT	CTGCTGCTTG	CAAACAAAAA	AACCACCGCT	ACCAGCGGTG	GTTTGTGTTG	2580
CGGATCAAGA	GCTACCAACT	CTTTTTCCGA	AGGTAAGTGG	CTTCAGCAGA	GCGCAGATAC	2640
CAAATACTGT	CCTTCTAGTG	TAGCCGTAGT	TAGGCCACCA	CTTCAAGAAC	TCTGTAGCAC	2700
CGCCTACATA	CCTCGCTCTG	CTAATCCTGT	TACCAGTGGC	TGCTGCCAGT	GCGGATAAGT	2760
CGTGTCTTAC	CGGGTTGGAC	TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	CGGTCGGGCT	2820
GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	GACCTACACC	GAAGTGTGAT	2880
ACCTACAGCG	TGAGCTATGA	GAAAGCGCCA	CGCTTCCCGA	AGGGAGAAAG	GCGGACAGGT	2940
ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG	AGCGCACGAG	GGAGCTTCCA	GGGGGAAACG	3000
CCTGGTATCT	TTATAGTCCT	GTCGGGTTTC	GCCACCTCTG	ACTTGAGCGT	CGATTTTGT	3060
GATGCTCGTC	AGGGGGGCGG	AGCCTATGGA	AAAACGCCAG	CAACGCGGCC	TTTTTACGGT	3120
TCCTGGCCTT	TTGCTGGCCT	TTTGCTCACA	TGTTCTTTCC	TGCGTTATCC	CCTGATTCTG	3180
TGGATAACCG	TATTACCGCC	TTTGAGTGAG	CTGATACCGC	TCGCCGCAGC	CGAACGACCG	3240
AGCGCAGCGA	GTCAGTGAGC	GAGGAAGCGG	AAGAGCGCCT	GATGCGGTAT	TTTCTCCTTA	3300
CGCATCTGTG	CGGTATTTCA	CACCGCATAT	GGTGCACCTC	CAGTACAATC	TGCTCTGATG	3360
CCGCATAGTT	AAGCCAGTAT	ACACTCCGCT	ATCGCTACGT	GACTGGGTCA	TGGCTGCGCC	3420
CCGACACCCG	CCAACACCCG	CTGACGCGCC	CTGACGGGCT	TGTCTGCTCC	CGGCATCCGC	3480
TTACAGACAA	GCTGTGACCG	TCTCCGGGAG	CTGCATGTGT	CAGAGGTTTT	CACCGTCATC	3540
ACCGAAACGC	GCGAGGCAGC	AGATCAATTC	GCGCGCGAAG	GCGAAGCGGC	ATGCATAATG	3600
TGCTGTCAA	ATGGACGAAG	CAGGGATTCT	GCAAACCCTA	TGCTACTCCG	TCAAGCCGTC	3660
AATTGTCTGA	TTCGTTACCA	ATTATGACAA	CTTGACGGCT	ACATCATTTA	CTTTTTCTTC	3720
ACAACCGGCA	CGGAACCTGC	TCGGGCTGGC	CCCGGTGCAT	TTTTTAAATA	CCCGCGAGAA	3780
ATAGAGTTGA	TCGTCAAAAC	CAACATTGCG	ACCGACGGTG	GCGATAGGCA	TCCGGGTGGT	3840
GCTCAAAAGC	AGCTTCGCCT	GGCTGATACG	TTGGTCCTCG	CGCCAGCTTA	AGACGCTAAT	3900
CCCTAACTGC	TGGCGGAAAA	GATGTGACAG	ACGCGACGGC	GACAAGCAAA	CATGCTGTGC	3960
GACGCTGGCG	ATATCAAAAT	TGCTGTCTGC	CAGGTGATCG	CTGATGTACT	GACAAGCCTC	4020
GCGTACCCGA	TTATCCATCG	GTGGATGGAG	CGACTCGTTA	ATCGCTTCCA	TGCGCCGCAG	4080
TAACAATTGC	TCAAGCAGAT	TTATCGCCAG	CAGCTCCGAA	TAGCGCCCTT	CCCCTTGCCC	4140
GGCGTTAATG	ATTTGCCCAA	ACAGGTGCGT	GAAATGCGGC	TGGTGCGCTT	CATCCGGGCG	4200
AAAGAACCCC	GTATTGGCAA	ATATTGACGG	CCAGTTAAGC	CATTCATGCC	AGTAGGCGCG	4260
CGGACGAAAG	TAAACCCACT	GGTGATACCA	TTCGCGAGCC	TCCGGATGAC	GACCGTAGTG	4320
ATGAATCTCT	CCTGGCGGGA	ACAGCAAAAT	ATCACCCGGT	CGGCAAAACA	ATTCTCGTCC	4380
CTGATTTTTT	ACCACCCCTT	GACCGCGAAT	GGTGAGATTG	AGAATATAAC	CTTTCATTCC	4440
CAGCGGTCTG	TCGATAAAAA	AATCGAGATA	ACCGTTGGCC	TCAATCGGCG	TTAAACCCGC	4500
CACCAGATGG	GCATTAAACG	AGTATCCCGG	CAGCAGGGGA	TCATTTTGCG	CTTCAGCCAT	4560
ACTTTTCATA	CTCCCGCCAT	TCAGAGAAGA	AACCAATTGT	CCATATTGCA	TCAGACATTG	4620
CCGTCACTGC	GTCTTTTACT	GGCTCTTCTC	GCTAACCAAA	CCGGTAACCC	CGCTTATTAA	4680
AAGCATTCTG	TAACAAAGCG	GGACCAAGC	CATGACAAAA	ACGCGTAACA	AAAGTGTCTA	4740
TAATCACGGC	AGAAAAGTCC	ACATTGATTA	TTTGCACGGC	GTCACACTTT	GCTATGCCAT	4800
AGCATTTTTA	TCCATAAGAT	TAGCGGATCC	TACCTGACGC	TTTTTATCGC	AACTCTCTAC	4860
TGTTTCTCCA	TACCCGTTTT	TTTGGGCTAA	CAGGAGGAAT	TAACCATGGG	GGGTTCTCAT	4920
CATCATCATC	ATCATGGTAT	GGCTAGCATG	ACTGGTGGAC	AGCAAATGGG	TCGGGATCTG	4980
TACGACGATG	ACGATAAGGA	TCGATGGGGA	TCCGAGCTCG	A		5021

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FIG. 18.



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FIG. 19

PGL2control (promega)

1 GGTACCGAGC TCTTACGCGT GCTAGCCCGG GCTCGAGATC TGCGATCTGC
CCATGGCTCG AGAATGCGCA CGATCGGGCC CGAGCTCTAG ACGCTAGACG

51 ATCTCAATTA GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCCG
TAGAGTTAAT CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC

101 CCCCTAACTC CGCCAGTTC CGCCATTCT CCGCCCCATC GCTGACTAAT
GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAG CGACTGATTA

151 TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC
AAAAAATAA ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG

201 AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAGCTT
TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAA

251 GGCATTCCGG TACTGTTGGT AAAGCCACCA TGGAAGACGC CAAAACATA
CCGTAAGGCC ATGACAACCA TTTCGGTGGT ACCTTCTGCG GTTTTTGTAT

301 AAGAAAGGCC CGGCGCCATT CTATCCGCTG GAAGATGGAA CCGCTGGAGA
TTCTTTCCGG GCCGCGGTAA GATAGGCGAC CTTCTACCTT GGCGACCTCT

351 GCAACTGCAT AAGGCTATGA AGAGATACGC CCTGGTTCCT GGAACAATTG
CGTTGACGTA TTCCGATACT TCTCTATGCG GGACCAAGGA CCTTGTTAAC

401 CTTTTACAGA TGCACATATC GAGGTGGACA TCACTTACGC TGAGTACTTC
GAAATGTCT ACGTGTATAG CTCCACCTGT AGTGAATGCG ACTCATGAAG

451 GAAATGTCCG TTCGGTTGGC AGAAGCTATG AAACGATATG GGCTGAATAC
CTTTACAGGC AAGCCAACCG TCTTCGATAC TTTGCTATAC CCGACTTATG

501 AAATCACAGA ATCGTCGTAT GCAGTGAAAA CTCTCTTCAA TTCTTTATGC
TTTAGTGTCT TAGCAGCATA CGTCACTTTT GAGAGAAGTT AAGAAATACG

551 CGGTGTTGGG CGCGTTATTT ATCGGAGTTG CAGTTGCGCC CGCGAACGAC
GCCACAACCC GCGCAATAAA TAGCCTCAAC GTCAACGCGG GCGCTTGCTG

601 ATTTATAATG AACGTGAATT GCTCAACAGT ATGGGCATTT CGCAGCCTAC
TAAATATTAC TTGCACTTAA CGAGTTGTCA TACCCGTAAA GCGTCGGATG

651 CGTGGTGTTT GTTTCCAAAA AGGGGTTGCA AAAAATTTTG AACGTGCAAA
GCACCACAAG CAAAGGTTTT TCCCCAACGT TTTTAAAC TTGCACGTTT

701 AAAAGCTCCC AATCATCCAA AAAATTATTA TCATGGATTC TAAAACGGAT
TTTTCGAGGG TTAGTAGGTT TTTTAATAAT AGTACCTAAG ATTTTGCCTA

751 TACCAGGGAT TTCAGTCGAT GTACACGTTT GTCACATCTC ATCTACCTCC
ATGGTCCCTA AAGTCAGCTA CATGTGCAAG CAGTGTAGAG TAGATGGAGG

801 CGGTTTTAAT GAATACGATT TTGTGCCAGA GTCCTTCGAT AGGGACAAGA
GCCAAAATTA CTTATGCTAA AACACGGTCT CAGGAAGCTA TCCCTGTTCT

851 CAATTGCACT GATCATGAAC TCCTCTGGAT CTAAGGTCT GCCTAAAGGT
GTTAACGTGA CTAGTACTTG AGGAGACCTA GATGACCAGA CGGATTTC

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FIG. 19. (CONTINUED)

901 GTCGCTCTGC CTCATAGAAC TGCCTGCGTG AGATTCTCGC ATGCCAGAGA
CAGCGAGACG GAGTATCTTG ACGGACGCAC TCTAAGAGCG TACGGTCTCT

951 TCCTATTTTT GGCAATCAAA TCATTCCGGA TACTGCGATT TTAAGTGTTG
AGGATAAAAA CCGTTAGTTT AGTAAGGCCT ATGACGCTAA AATTCACAAC

1001 TTCCATTCCA TCACGGTTTT GGAATGTTTA CTACACTCGG ATATTTGATA
AAGGTAAGGT AGTGCCAAAA CCTTACAAAT GATGTGAGCC TATAAACTAT

1051 TGTGGATTTC GAGTCGTCTT AATGTATAGA TTTGAAGAAG AGCTGTTTCT
ACACCTAAAG CTCAGCAGAA TTACATATCT AAACCTTCTC TCGACAAAGA

1101 GAGGAGCCTT CAGGATTACA AGATTCAAAG TCGCTGCTG GTGCCAACCC
CTCCTCGGAA GTCCTAATGT TCTAAGTTTC ACGCGACGAC CACGGTTGGG

1151 TATTCTCCTT CTTGCGCCAA AGCACTCTGA TTGACAAATA CGATTTATCT
ATAAGAGGAA GAAGCGGTTT TCGTGAGACT AACTGTTTAT GCTAAATAGA

1201 AATTTACACG AAATTGCTTC TGGTGGCGCT CCCCTCTCTA AGGAAGTCGG
TTAAATGTGC TTAAACGAAG ACCACCGCGA GGGGAGAGAT TCCTTCAGCC

1251 GGAAGCGGTT GCCAAGAGGT TCCATCTGCC AGGTATCAGG CAAGGATATG
CCTTCGCCAA CGGTTCTCCA AGGTAGACGG TCCATAGTCC GTTCCTATAC

1301 GGCTCACTGA GACTACATCA GCTATTCTGA TTACACCCGA GGGGGATGAT
CCGAGTGACT CTGATGTAGT CGATAAGACT AATGTGGGCT CCCCTACTA

1351 AAACCGGGCG CGGTCGGTAA AGTTGTTCCA TTTTTTGAAG CGAAGGTTGT
TTTGGCCCGC GCCAGCCATT TCAACAAGGT AAAAACTTC GCTTCCAACA

1401 GGATCTGGAT ACCGGGAAAA CGCTGGGCGT TAATCAAAGA GGCGAACTGT
CCTAGACCTA TGGCCCTTTT GCGACCCGCA ATTAGTTTCT CCGCTTGACA

1451 GTGTGAGAGG TCCTATGATT ATGTCCGGTT ATGTAAACAA TCCGGAAGCG
CACACTCTCC AGGATACTAA TACAGGCCAA TACATTTGTT AGGCCTTCGC

1501 ACCAACGCCT TGATTGACAA GGATGGATGG CTACATTCTG GAGACATAGC
TGGTTGCGGA ACTAACTGTT CCTACCTACC GATGTAAGAC CTCTGTATCG

1551 TTAAGTGGAC GAAGACGAAC ACTTCTTCAT CGTTGACCGC CTGAAGTCTC
AATGACCCTG CTTCTGCTTG TGAAGAAGTA GCAACTGGCG GACTTCAGAG

1601 TGATTAAGTA CAAAGGCTAT CAGGTGGCTC CCGCTGAATT GGAATCCATC
ACTAATTCA TTTTCCGATA GTCCACCGAG GGCGACTTAA CCTTAGGTAG

1651 TTGCTCCAAC ACCCCAACAT CTTGACGCA GGTGTCGCAG GTCTTCCCGA
AACGAGGTTG TGGGGTTGTA GAAGCTGCGT CCACAGCGTC CAGAAGGGCT

1701 CGATGACGCC GGTGAACTTC CCGCCGCCGT TGTTGTTTTG GAGCACGGAA
GCTACTGCGG CCACTTGAAG GGCGGCGGCA ACAACAAAAC CTCGTGCCTT

1751 AGACGATGAC GGAAAAAGAG ATCGTGGATT ACGTCGCCAG TCAAGTAACA
TCTGCTACTG CCTTTTTCTC TAGCACCTAA TGCAGCGGTC AGTTCATTGT

1801 ACCGCGAAAA AGTTGCGCGG AGGAGTTGTG TTTGTGGACG AAGTACCGAA
TGGCGCTTTT TCAACGCGCC TCCTCAACAC AAACACCTGC TTCATGGCTT

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FIG. 19. (CONTINUED)

1851 AGGTCTTACC GGAAAACTCG ACGCAAGAAA AATCAGAGAG ATCCTCATAA
TCCAGAATGG CCTTTTGAGC TCGGTTCTTT TTAGTCTCTC TAGGAGTATT

1901 AGGCCAAGAA GGGCGGAAAG ATCGCCGTGT AATTCTAGAG TCGGGGCGGC
TCCGGTTCTT CCCGCCTTTC TAGCGGCACA TTAAGATCTC AGCCCCGCCG

1951 CGGCCGCTTC GAGCAGACAT GATAAGATAC ATTGATGAGT TTGGACAAAC
GCCGGCGAAG CTCGTCTGTA CTATTCTATG TAACTACTCA AACCTGTTTG

2001 CACAACTAGA ATGCAGTGAA AAAAATGCTT TATTTGTGAA ATTTGTGATG
GTGTTGATCT TACGTCACCT TTTTACGAA ATAAACACTT TAAACACTAC

2051 CTATTGCTTT APTTGTAACC ATTATAAGCT GCAATAAACA AGTTAACAAC
GATAACGAAA TAAACATTGG TAATATTCGA CGTTATTTGT TCAATTGTTG

2101 AACAAATTGCA TTCATTTTAT GTTTCAGGTT CAGGGGGAGG TGTGGGAGGT
TTGTAAACGT AAGTAAAATA CAAAGTCCAA GTCCCCCTCC ACACCCTCCA

2151 TTTTAAAGC AAGTAAACC TCTACAAATG TGGTAAATC GATAAGGATC
AAAAATTTCC TTCATTTTGG AGATGTTTAC ACCATTTTAG CTATTCCTAG

2201 TGAACGATGG AGCGGAGAAT GGGCGGAACT GGGCGGAGTT AGGGGCGGGA
ACTTGCTACC TCGCCTCTTA CCCGCCTTGA CCCGCCTCAA TCCCCGCCCT

2251 TGGGCGGAGT TAGGGGCGGG ACTATGGTTG CTGACTAATT GAGATGCATG
ACCCGCCTCA ATCCCCGCC TGATACCAAC GACTGATTAA CTCTACGTAC

2301 CTTTGCATAC TTCTGCCTGC TGGGGAGCCT GGGGACTTTC CACACCTGGT
GAAACGTATG AAGACGGACG ACCCCTCGGA CCCCTGAAAG GTGTGGACCA

2351 TGCTGACTAA TTGAGATGCA TGCTTTGCAT ACTTCTGCCT GCTGGGGAGC
ACGACTGATT AACTCTACGT ACGAAACGTA TGAAGACGGA CGACCCCTCG

2401 CTGGGGACTT TCCACACCCT AACTGACACA CATTCCACAG CGGATCCGTC
GACCCCTGAA AGGTGTGGGA TTGACTGTGT GTAAGGTGTC GCCTAGGCAG

2451 GACCGATGCC CTTGAGAGCC TTCAACCCAG TCAGCTCCTT CCGGTGGGCG
CTGGCTACGG GAACTCTCGG AAGTTGGGTC AGTCGAGGAA GGCCACCCGC

2501 CGGGGCATGA CTATCGTCGC CGCACTTATG ACTGTCTTCT TTATCATGCA
GCCCCGTACT GATAGCAGCG GCGTGAATAC TGACAGAAGA AATAGTACGT

2551 ACTCGTAGGA CAGGTGCCGG CAGCGCTCTT CCGCTTCCTC GCTCACTGAC
TGAGCATCCT GTCCACGGCC GTCGCGAGAA GCGGAAGGAG CGAGTGACTG

2601 TCGCTGCGCT CCGTCGTTTC GCTGCGGCGA GCGGTATCAG CTCACTCAA
AGCGACGCGA GCCAGCAAGC CGACGCCGCT CGCCATAGTC GAGTGAGTTT

2651 GGCGGTAATA CGGTTATCCA CAGAATCAGG GGATAACGCA GGAAAGAACA
CCGCCATTAT GCCAATAGGT GTCTTAGTCC CCTATTGCGT CCTTTCTTGT

2701 TGTGAGCAAA AGGCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG
ACACTCGTTT TCCGGTCGTT TTCCGGTCCT TGGCATTTTT CCGGCGCAAC

2751 CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC AAAAAATCG
GACCGCAAAA AAGTATCCGA GCGGGGGGGA CTGCTCGTAG TGTTTTTCAGC

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FIG. 19. (CONTINUED)

2801 ACGCTCAAGT CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG
TGCGAGTTCA GTCTCCACCG CTTTGGGCTG TCCTGATATT TCTATGGTCC

2851 CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC GACCCTGCCG
GCAAAGGGGG ACCTTCGAGG GAGCACGCGA GAGGACAAGG CTGGGACGGC

2901 CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC
GAATGGCCTA TGGACAGGCG GAAAGAGGGA AGCCCTTCGC ACCGCGAAAG

2951 TCAATGCTCA CGCTGTAGGT ATCTCAGTTC GGTGTAGGTC GTTCGCTCCA
AGTTACGAGT GCGACATCCA TAGAGTCAAG CCACATCCAG CAAGCGAGGT

3001 AGCTGGGCTG TGTGCACGAA CCCCCGTTT AGCCCGACCG CTGCGCCTTA
TCGACCCGAC ACACGTGCTT GGGGGGCAAG TCGGGCTGGC GACGCGGAAT

3051 TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC
AGGCCATTGA TAGCAGAACT CAGGTTGGGC CATTCTGTGC TGAATAGCGG

3101 ACTGGCAGCA GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG
TGACCGTCGT CGGTGACCAT TGTCTAATC GTCTCGCTCC ATACATCCGC

3151 GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGG
CACGATGTCT CAAGAACTTC ACCACCGGAT TGATGCCGAT GTGATCTTCC

3201 ACAGTATTTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT TCGGAAAAAG
TGTCATAAAC CATAGACGCG AGACGACTTC GGTCAATGGA AGCCTTTTTTC

3251 AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT
TCAACCATCG AGAACTAGGC CGTTTGTTTG GTGGCGACCA TCGCCACCAA

3301 TTTTGTGTTG CAAGCAGCAG ATTACGCGCA GAAAAAAGG ATCTCAAGAA
AAAAACAAAC GTTCGTCTGC TAATGCGCGT CTTTTTTTCC TAGAGTTCTT

3351 GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAACTC
CTAGGAAACT AGAAAAGATG CCCCAGACTG CGAGTCACCT TGCTTTTGAG

3401 ACGTTAAGGG ATTTTGGTCA TGAGATTATC AAAAAGGATC TTCACCTAGA
TGCAATTCCC TAAACACAGT ACTCTAATAG TTTTTCCTAG AAGTGGATCT

3451 TCCTTTTAAA TTAAAAATGA AGTTTAAAT CAATCTAAAG TATATATGAG
AGGAAAATTT AATTTTACT TCAAAATTTA GTTAGATTTT ATATATACTC

3501 TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG CACCTATCTC
ATTTGAACCA GACTGTCAAT GGTTACGAAT TAGTCACCTC GTGGATAGAG

3551 AGCGATCTGT CTATTTCTGT CATCCATAGT TGCCTGACTC CCCGTCGTGT
TCGCTAGACA GATAAAGCAA GTAGGTATCA ACGGACTGAG GGGCAGCACA

3601 AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG
TCTATTGATG CTATGCCCTC CCGAATGGTA GACCGGGGTC ACGACGTTAC

3651 ATACCGCGAG ACCCAGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA
TATGGCGCTC TGGGTGCGAG TGGCCGAGGT CTAAATAGTC GTTATTTGGT

3701 GCCAGCCGGA AAGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT
CGGTCTGGCT TCCCGGCTCG CGTCTTCACC AGGACGTTGA AATAGGCGGA

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FIG. 19. (CONTINUED)

3751 CCATCCAGTC TATTAATTGT TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA
GGTAGGTCAG ATAATTAACA ACGGCCCTTC GATCTCATTC ATCAAGCGGT

3801 GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTACAGGCA TCGTGGTGTC
CAATTATCAA ACGCGTTGCA ACAACGGTAA CGATGTCCGT AGCACCACAG

3851 ACGCTCGTCG TTTGGTATGG CTTCAATTCAG CTCCGGTTCC CAACGATCAA
TGCGAGCAGC AAACCATAACC GAAGTAAGTC GAGGCCAAGG GTTGCTAGTT

3901 GCGGAGTTAC ATGATCCCCC ATGTTGTGCA AAAAAGCGGT TAGCTCCTTC
CCGCTCAATG TACTAGGGGG TACAACACGT TTTTTCGCCA ATCGAGGAAG

3951 GGTCTCCGA TCGTTGTCAG AAGTAAGTTG GCCGCAGTGT TATCACTCAT
CCAGGAGGCT AGCAACAGTC TTCATTCAAC CGGCGTCACA ATAGTGAGTA

4001 GGTTATGGCA GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT
CCAATACCGT CGTGACGTAT TAAGAGAATG ACAGTACGGT AGGCATTCTA

4051 GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT
CGAAAAGACA CTGACCACTC ATGAGTTGGT TCAGTAAGAC TCTTATCACA

4101 ATGCGGCGAC CGAGTTGCTC TTGCCCCGGC TCAATACGGG ATAATACCGC
TACGCCGCTG GCTCAACGAG AACGGGCCGC AGTTATGCCC TATTATGGCG

4151 GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG
CGGTGTATCG TCTTGAAATT TTCACGAGTA GTAACCTTTT GCAAGAAGCC

4201 GCGGAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA
CCGCTTTTGA GAGTTCCTAG AATGGCGACA ACTCTAGGTC AAGCTACATT

4251 CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT
GGGTGAGCAC GTGGGTTGAC TAGAAGTCGT AGAAAATGAA AGTGGTCGCA

4301 TTCTGGGTGA GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA
AAGACCCACT CGTTTTTGTC CTTCCGTTTT ACGGCGTTTT TTCCCTTATT

4351 GGGCGACACG GAAATGTTGA ATACTCATACT TCTTCCTTTT TCAATATTAT
CCCGCTGTGC CTTTACAAC TATGAGTATG AGAAGGAAAA AGTTATAATA

4401 TGAAGCATT TATCAGGGTTA TTGTCTCATG AGCGGATACA TATTTGAATG
ACTTCGTAAA TAGTCCCAAT AACAGAGTAC TCGCCTATGT ATAAACTTAC

4451 TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCAGAAAG
ATAAATCTTT TTATTTGTTT ATCCCAAGG CGCGTGTAAG GGGGCTTTTC

4501 TGCCACCTGA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
ACGGTGGACT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

4551 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC
CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG

4601 TTTGCTTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCGGC TTTCCCGTTC
AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

4651 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG
TTCGAGATTT AGCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC

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FIG. 19. (CONTINUED).

4701 CACCTCGACC CCAAAAAACT TGATTAGGGT GATGGTTCAC GTAGTGGGCC
GTGGAGCTGG GGTTTTTTGA ACTAATCCCA CTACCAAGTG CATCACCCGG

4751 ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT
TAGCGGGACT ATCTGCCAAA AAGCGGGAAA CTGCAACCTC AGGTGCAAGA

4801 TTAATAGTGG ACTCTTGTTT CAAACTGGAA CAACACTCAA CCCTATCTCG
AATTATCACC TGAGAACAAG GTTTGACCTT GTTGTGAGTT GGGATAGAGC

4851 GTCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTTCGG CCTATTGGTT
CAGATAAGAA AACTAAATAT TCCCTAAAC GGCTAAAGCC GGATAACCAA

4901 AAAAAATGAG CTGATTTAAC AAAAATTTAA CGCGAATTTT AACAAAATAT
TTTTTTACTC GACTAAATTG TTTTAAATT GCGCTTAAA TTGTTTTATA

4951 TAACGTTTAC AATTTCCCAT TCGCCATTCA GGCTGCGCAA CTGTTGGGAA
ATTGCAAATG TTAAAGGGTA AGCGGTAAGT CCGACGCGTT GACAACCCTT

5001 GGGCGATCGG TCGGGGCCTC TTCGCTATTA CGCCAGCCCA AGCTACCATG
CCCGCTAGCC ACGCCCGGAG AAGCGATAAT GCGGTCGGGT TCGATGGTAC

5051 ATAAGTAAGT AATATTAAGG TACGGGAGGT ACTTGGAGCG GCCGCAATAA
TATTCATTCA TTATAATTCC ATGCCCTCCA TGAACCTCGC CGGCGTTATT

5101 AATATCTTTA TTTTCATTAC ATCTGTGTGT TGGTTTTTTG TGTGAATCGA
TTATAGAAAT AAAAGTAATG TAGACACACA ACCAAAAAC AACTTAGCT

5151 TAGTACTAAC ATACGCTCTC CATCAAAACA AAACGAAACA AAACAACTA
ATCATGATTG TATGCGAGAG GTAGTTTTGT TTTGCTTTGT TTTGTTTGAT

5201 GCAAAATAGG CTGTCCCCAG TGCAAGTGCA GGTGCCAGAA CATTTCTCTA
CGTTTTATCC GACAGGGGTC ACGTTCACGT CCACGGTCTT GTAAAGAGAT

5251 TCGATA
AGCTAT

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FIG. 20.

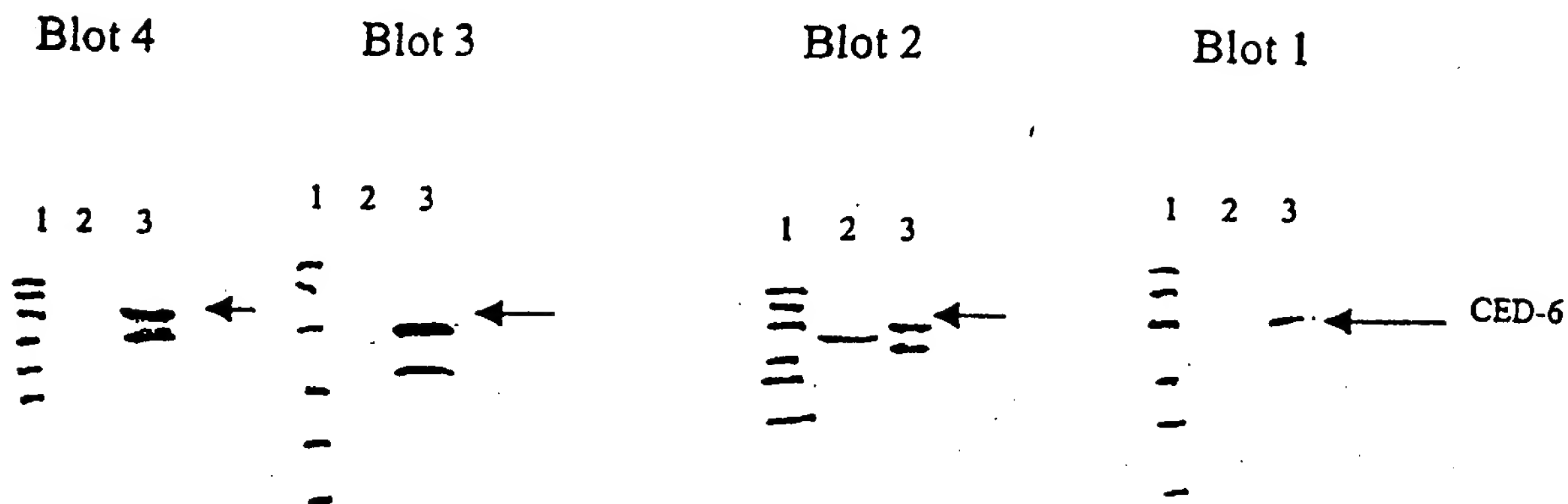


FIG. 21.

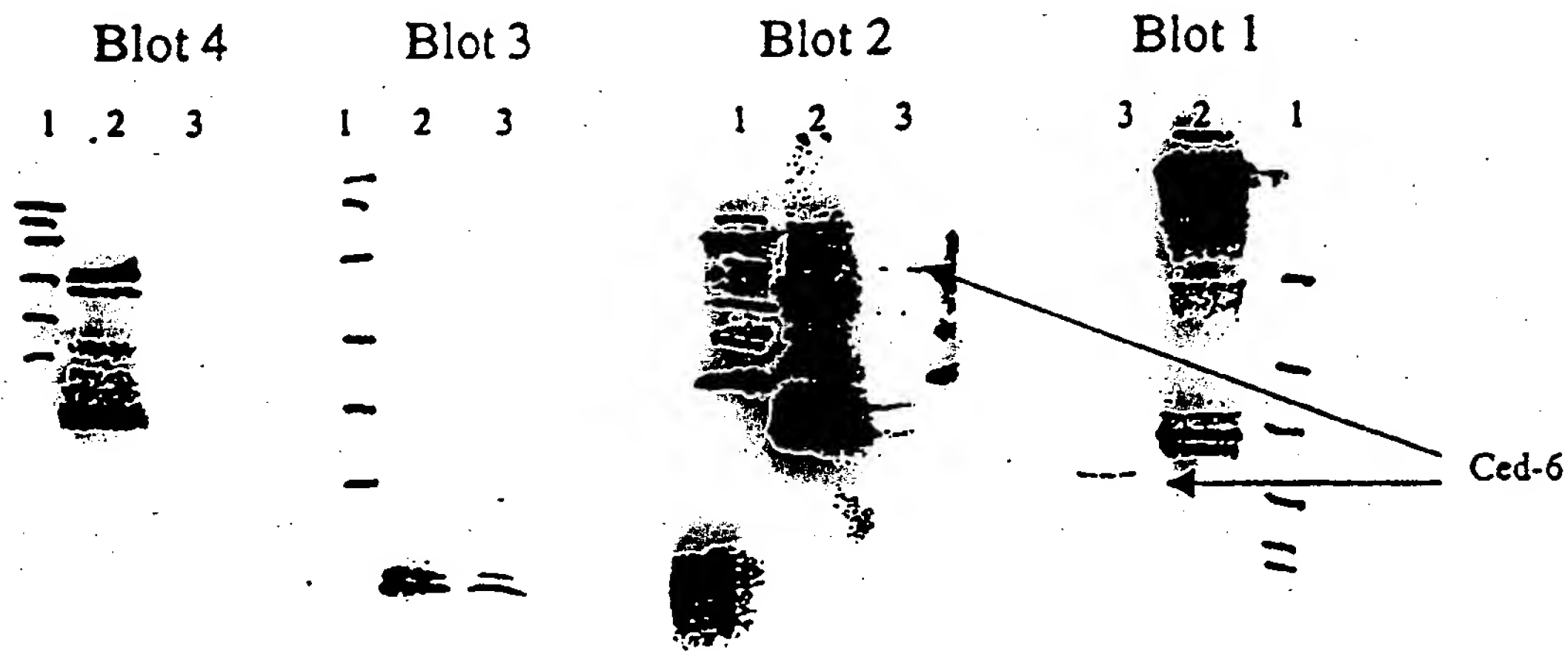
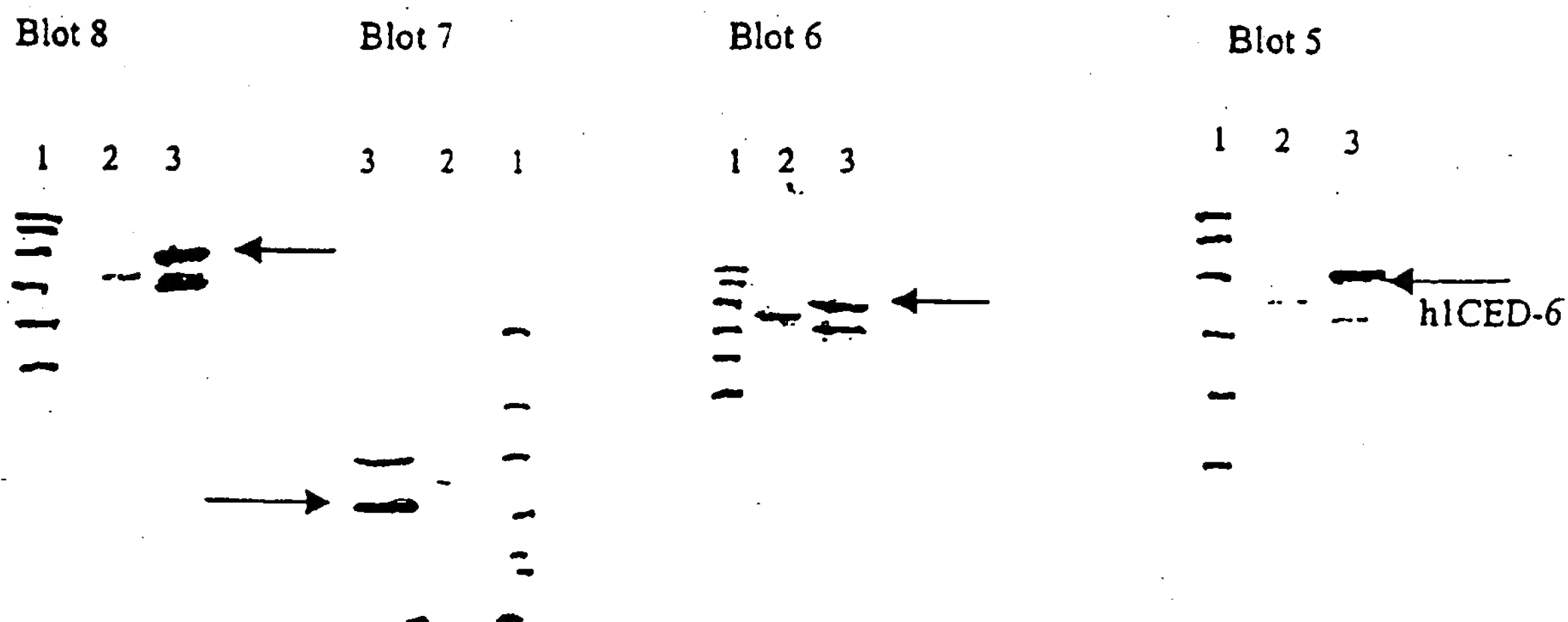


FIG. 22.



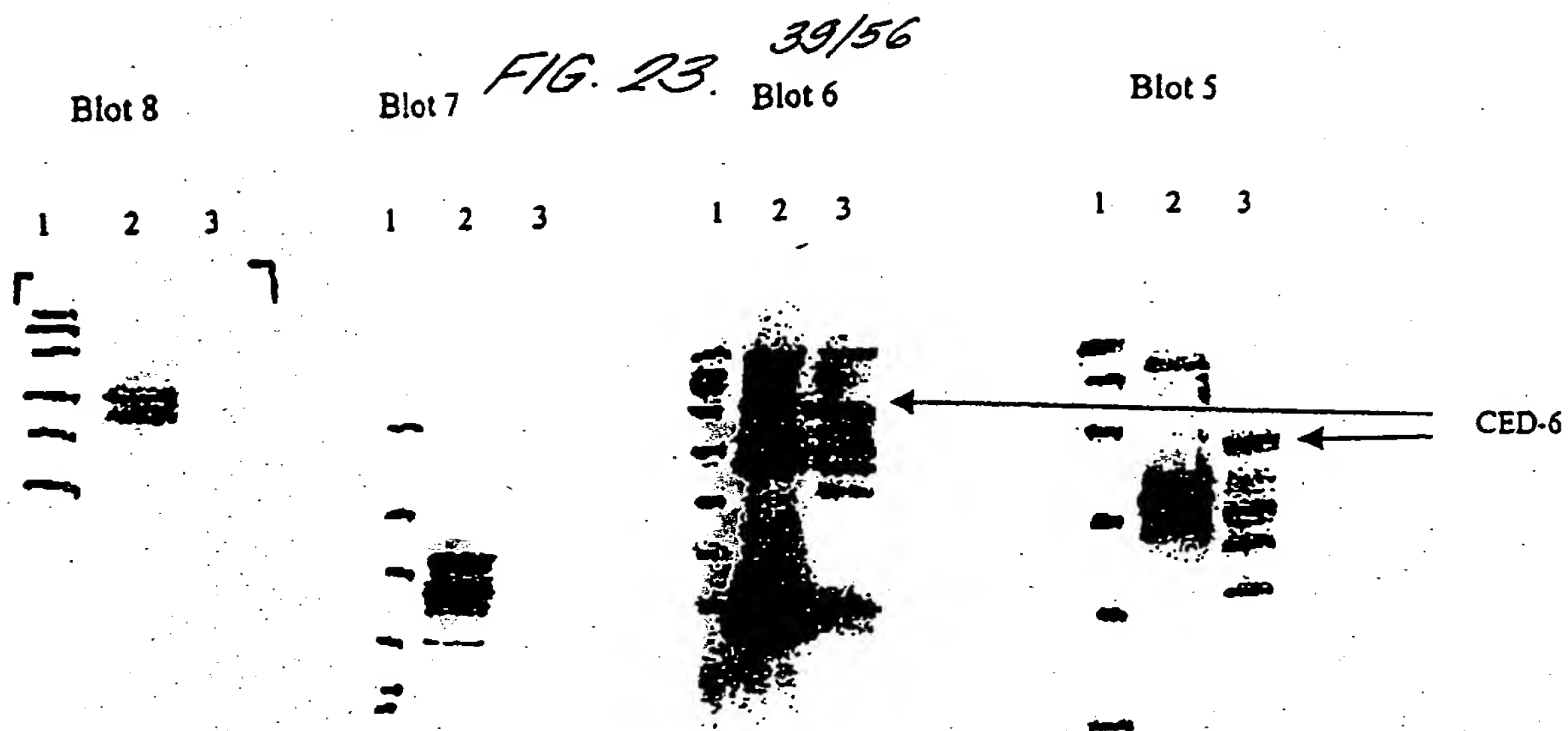


FIG. 24.

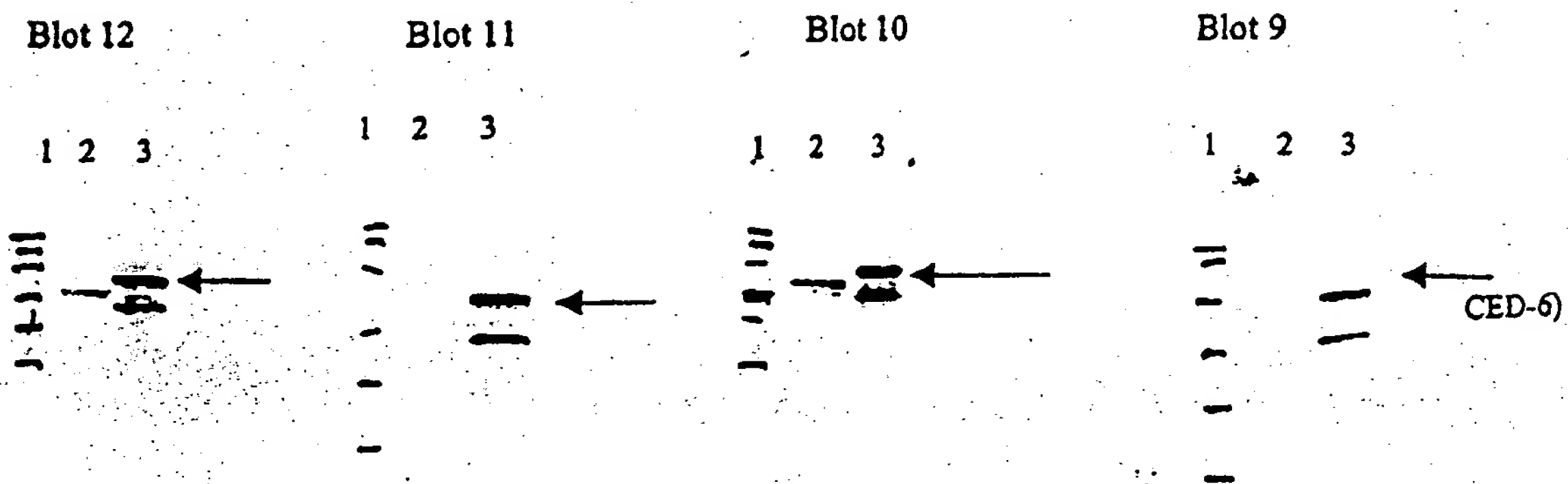
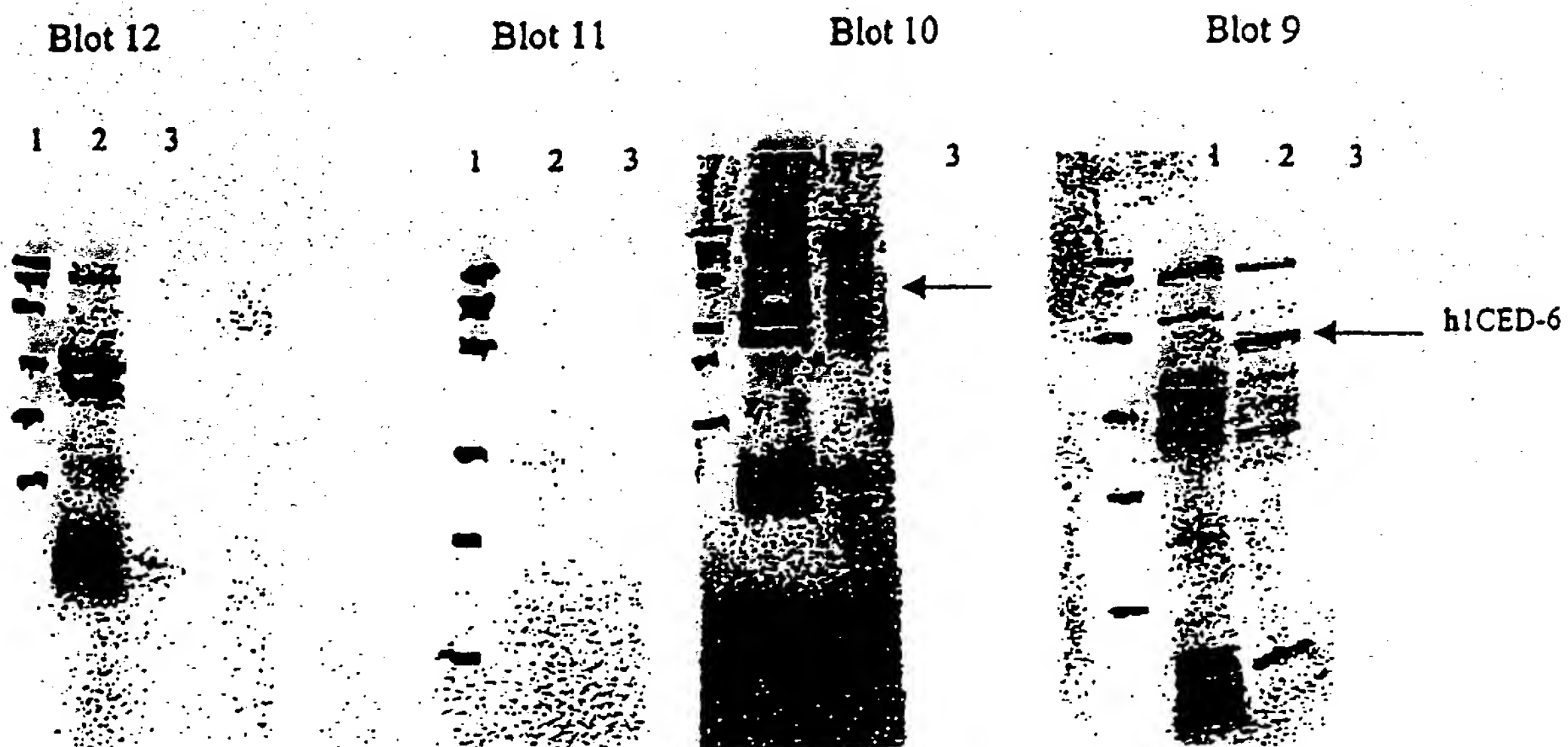


FIG. 25.



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FIG. 26.

SQ SEQUENCE 4735 BP
TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA TGGAGTTCCG
60
CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG CCCAACGACC CCCGCCCAT
120
GACGTCAATA ATGACGTATG TTCCCATAGT AACGCCAATA GGGACTTTCC ATTGACGTCA
180
ATGGGTGGAG TATTTACGGT AAAGTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC
240
AAGTACGCCC CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
300
CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA TCGCTATTAC
360
CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA TAGCGGTTTG ACTCACGGGG
420
ATTTCCAAGT CTCCACCCCA TTGACGTCAA TGGGAGTTTG TTTTGGCACC AAAATCAACG
480
GGACTTTCCA AAATGTCGTA ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT
540
ACGGTGGGAG GTCTATATAA GCAGAGCTGG TTTAGTGAAC CGTCAGATCC GCTAGCGCTA
600
CCGGTCGCCA CCATGGTGAG CAAGGGCGAG GAGCTGTTCA CCGGGGTGGT GCCCATCCTG
660
GTCGAGCTGG ACGGCGACGT AAACGGCCAC AAGTTCAGCG TGTCCGGCGA GGGCGAGGGC
720
GATGCCACCT ACGGCAAGCT GACCCTGAAG TTCATCTGCA CCACCGGCAA GCTGCCCCGTG
780
CCCTGGCCCA CCCTCGTGAC CACCCTGACC TACGGCGTGC AGTGCTTCAG CCGCTACCCC
840
GACCACATGA AGCAGCACGA CTTCTTCAAG TCCGCCATGC CCGAAGGCTA CGTCCAGGAG
900
CGCACCATCT TCTTCAAGGA CGACGGCAAC TACAAGACCC GCGCCGAGGT GAAGTTCGAG
960
GGCGACACCC TGGTGAACCG CATCGAGCTG AAGGGCATCG ACTTCAAGGA GGACGGCAAC
1020
ATCCTGGGGC ACAAGCTGGA GTACAACCTAC AACAGCCACA ACGTCTATAT CATGGCCGAC
1080
AAGCAGAAGA ACGGCATCAA GGTGAACTTC AAGATCCGCC ACAACATCGA GGACGGCAGC
1140
GTGCAGCTCG CCGACCACTA CCAGCAGAAC ACCCCCATCG GCGACGGCCC CGTGCTGCTG
1200
CCCGACAACC ACTACCTGAG CACCCAGTCC GCCCTGAGCA AAGACCCCAA CGAGAAGCGC
1260
GATCACATGG TCCGCTGGA GTTCGTGACC GCCGCCGGGA TCACTCTCGG CATGGACGAG
1320
CTGTACAAGT CCGGCCGGAC TCAGATCTCG AGCTCAAGCT TCGAATTCTG CAGTCGACGG
1380
TACCGCGGGC CCGGATCCA CCGGATCTAG ATAAGTATC ATAATCAGCC ATACCACATT
1440
TGTAAGAGTT TTAATTGCTT TAAAAACCT CCCACACCTC CCCCTGAACC TGAAACATAA
1500
AATGAATGCA ATTGTTGTTG TTAAGTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG
1560

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FIG. 26. (CONTINUED)

CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTTCA CTGCATTCTA GTTGTGGTTT
1620
GTCCAAACTC ATCAATGTAT CTTAACGCGT AAATTGTAAG CGTTAATATT TTGTTAAAAT
1680
TCGCGTTAAA TTTTGTGTTAA ATCAGCTCAT TTTTAAACCA ATAGGCCGAA ATCGGCAAAA
1740
TCCCTTATAA ATCAAAGAA TAGACCGAGA TAGGGTTGAG TGTGTGTTCCA GTTTGGAACA
1800
AGAGTCCACT ATTAAAGAAC GTGGACTCCA ACGTCAAAGG GCGAAAAACC GTCTATCAGG
1860
GCGATGGCCC ACTACGTGAA CCATCACCCT AATCAAGTTT TTTGGGGTCG AGGTGCCGTA
1920
AAGCACTAAA TCGGAACCCT AAAGGGAGCC CCCGATTTAG AGCTTGACGG GGAAAGCCGG
1980
CGAACGTGGC GAGAAAGGAA GGAAGAAAG CGAAAGGAGC GGGCGCTAGG GCGCTGGCAA
2040
GTGTAGCGGT CACGCTGCGC GTAACCACCA CACCCGCCGC GCTTAATGCG CCGCTACAGG
2100
GCGCGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGBAAC CCCTATTTGT TTATTTTCT
2160
AAATACATTC AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT
2220
ATTGAAAAAG GAAGAGTCCT GAGGCGGAAA GAACCAGCTG TGAATGTGT GTCAGTTAGG
2280
GTGTGGAAAG TCCCCAGGCT CCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
2340
GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT
2400
GCATCTCAAT TAGTCAGCAA CCATAGTCCC GCCCCTAACT CCGCCCATCC CGCCCCTAAC
2460
TCCGCCCAGT TCCGCCCAT TCCGCCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA
2520
GGCCGAGGCC GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTGGAGG
2580
CCTAGGCTTT TGCAAGATC GATCAAGAGA CAGGATGAGG ATCGTTTCGC ATGATTGAAC
2640
AAGATGGATT GCACGCAGGT TCTCCGGCCG CTGGGTGGA GAGGCTATTC GGCTATGACT
2700
GGGCACAACA GACAATCGGC TGCTCTGATG CCGCCGTGTT CCGGCTGTCA GCGCAGGGGC
2760
GCCCGGTTCT TTTGTCAAG ACCGACCTGT CCGGTGCCCT GAATGAACTG CAAGACGAGG
2820
CAGCGCGGCT ATCGTGGCTG GCCACGACGG GCGTTCCTTG CGCAGCTGTG CTCGACGTTG
2880
TCACTGAAGC GGAAGGGAC TGGCTGCTAT TGGGCGAAGT GCCGGGGCAG GATCTCCTGT
2940
CATCTCACCT TGCTCCTGCC GAGAAAGTAT CCATCATGGC TGATGCAATG CGGCGGCTGC
3000
ATACGCTTGA TCCGGCTACC TGCCCATTCG ACCACCAAGC GAAACATCGC ATCGAGCGAG
3060
CACGTAICTG GATGGAAGCC GGTCTTGTCG ATCAGGATGA TCTGGACGAA GAGCATCAGG
3120
GGCTCGCGCC AGCCGAAGT TCGCCAGGC TCAAGGCGAG CATGCCCGAC GCGAGGATC
3180
TCGTCGTGAC CCAAGGCGAT GCCTGCTTGC CGAATATCAT GGTGGAAAAT GGCCGCTTTT
3240

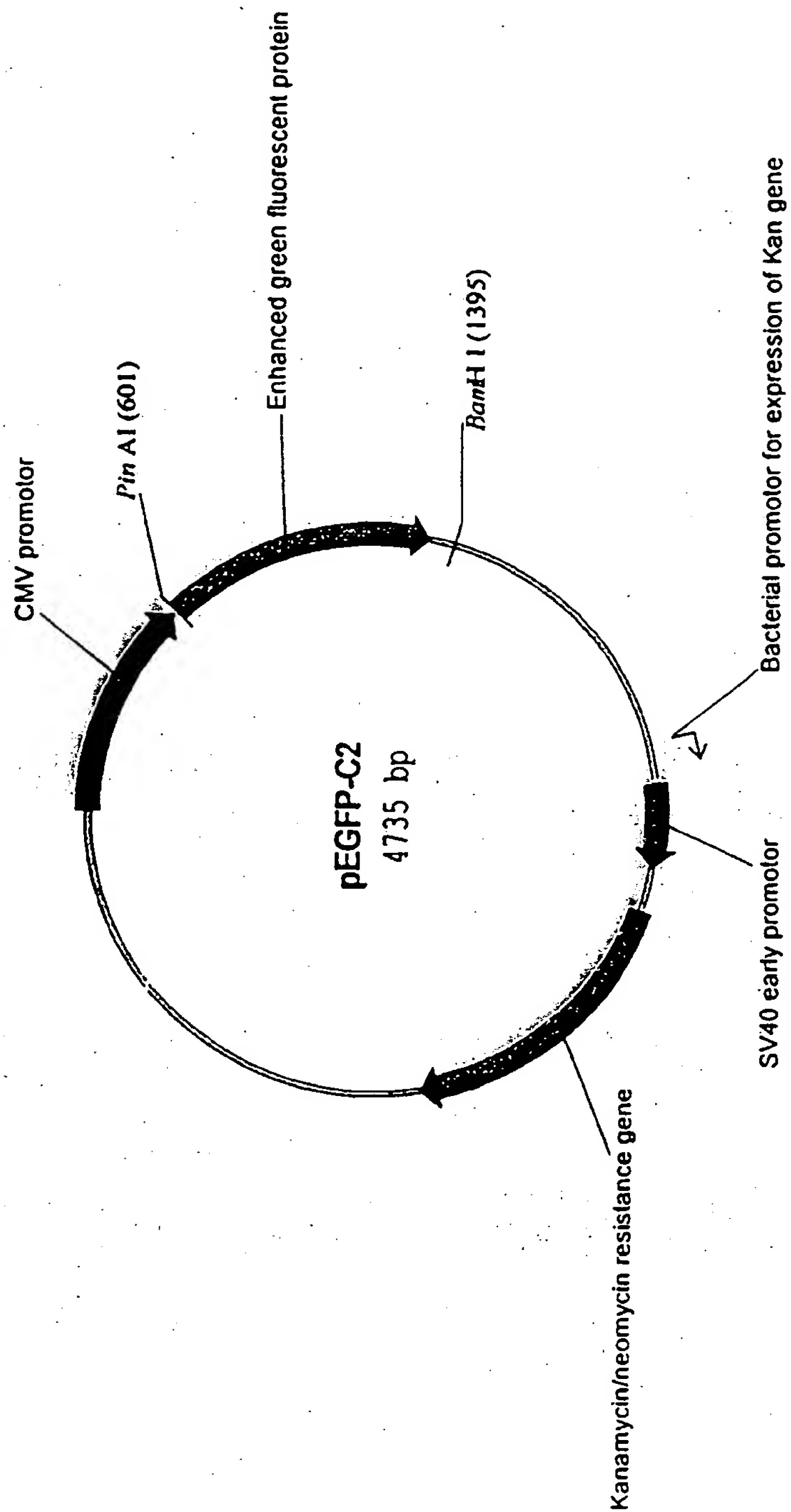
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FIG. 26. (CONTINUED)

CTGGATTTCAT CGACTGTGGC CGGCTGGGTG TGGCGGACCG CTATCAGGAC ATAGCGTTGG
3300 CTACCCGTGA TATTGCTGAA GAGCTTGGCG GCGAATGGGC TGACCGCTTC CTCGTGCTTT
3360 ACGGTATCGC CGCTCCCGAT TCGCAGCGCA TCGCCTTCTA TCGCCTTCTT GACGAGTTCT
3420 TCTGAGCGGG ACTCTGGGGT TCGAAATGAC CGACCAAGCG ACGCCCAACC TGCCATCACG
3480 AGATTTTCGAT TCCACCGCCG CCTTCTATGA AAGGTTGGGC TTCGGAATCG TTTTCCGGGA
3540 CGCCGGCTGG ATGATCCTCC AGCGCGGGGA TCTCATGCTG GAGTTCTTCG CCCACCCTAG
3600 GGGGAGGCTA ACTGAAACAC GGAAGGAGAC AATACCGGAA GGAACCCGCG CTATGACGGC
3660 AATAAAAAGA CAGAATAAAA CGCACGGTGT TGGGTCGTTT GTTCATAAAC GCGGGGTTCG
3720 GTCCCAGGGC TGGCACTCTG TCGATACCCC ACCGAGACCC CATTGGGGCC AATACGCCCC
3780 CGTTTCTTCC TTTTCCCCAC CCCACCCCCC AAGTTCGGGT GAAGGCCAG GGCTCGCAGC
3840 CAACGTCGGG GCGGCAGGCC CTGCCATAGC CTCAGGTTAC TCATATATAC TTTAGATTGA
3900 TTTAAACTT CATTTTAAAT TTAAAGGAT CTAGGTGAAG ATCCTTTTTG ATAATCTCAT
3960 GACCAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT
4020 CAAAGGATCT TCTTGAGATC CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAA
4080 ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTCCGAA
4140 GGTAAGTGGC TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT
4200 AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC TAATCCTGTT
4260 ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA
4320 GTTACCGGAT AAGGCGCAGC GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT
4380 GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC
4440 GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA
4500 GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG TCGGGTTTCG
4560 CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA
4620 AAACGCCAGC AACGCGGCCT TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT
4680 GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCA TGCAT
4735
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FIG. 27



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FIG. 28.

SQ SEQUENCE 5628 BP
TCGACGGTAC CGCGGGCCCG GGATCCACCG GATCTAGATA ACTGATCATA ATCAGCCATA
60 CCACATTTGT AGAGGTTTAA CTTGCTTTAA AAAACCTCCC ACACCTCCCC CTGAACCTGA
120 AACATAAAAT GAATGCAATT GTTGTTGTTA ACTTGTTTAT TGCAGCTTAT AATGGTTACA
180 AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCCTG CATTCTAGTT
240 GTGGTTTGTC CAAACTCATC AATGTATCTT AACGCGTAAA TTGTAAGCGT TAATATTTTG
300 TTAAAATTCG CGTTAAATTT TTGTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC
360 GGCAAAATCC CTTATAAATC AAAAGAATAG ACCGAGATAG GGTGAGTGT TGTTCAGTT
420 TGGAACAAGA GTCCACTATT AAAGAACGTG GACTCCAACG TCAAAGGGCG AAAAACCGTC
480 TATCAGGGCG ATGGCCCACT ACGTGAACCA TCACCCTAAT CAAGTTTTTT GGGGTCGAGG
540 TGCCGTAAAG CACTAAATCG GAACCCTAAA GGGAGCCCCC GATTAGAGC TTGACGGGGA
600 AAGCCGGCGA ACGTGGCGAG AAAGGAAGGG AAGAAAGCGA AAGGAGCGGG CGCTAGGGCG
660 CTGGCAAGTG TAGCGGTCAC GCTGCGCGTA ACCACCACAC CCGCCGCGCT TAATGCGCCG
720 CTACAGGGCG CGTCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA
780 TTTTCTAAA TACATTCAA TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT
840 CAATAATATT GAAAAGGAA GAGTCCTGAG GCGGAAAGAA CCAGCTGTGG AATGTGTGTC
900 AGTTAGGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC
960 TCAATTAGTC AGCAACCAGG TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC
1020 AAAGCATGCA TCTCAATTAG TCAGCAACCA TAGTCCCGCC CCTAACTCCG CCCATCCCGC
1080 CCCTAACTCC GCCCAGTTCC GCCCATTCTC CGCCCATGG CTGACTAATT TTTTTATTT
1140 ATGCAGAGGC CGAGGCCGCC TCGGCCTCTG AGCTATTCCA GAAGTAGTGA GGAGGCTTTT
1200 TTGGAGGCCT AGGCTTTTGC AAAGATCGAT CAAGAGACAG GATGAGGATC GTTTCGCATG
1260 ATTGAACAAG ATGGATTGCA CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTCGGC
1320 TATGACTGGG CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCCG GCTGTCAGCG
1380 CAGGGGCGCC CGGTTCTTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAAGTCAA
1440 GACGAGGCAG CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC AGCTGTGCTC
1500

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FIG. 28 (CONTINUED)

1560 GACGTTGTCA CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT
CTCCTGTCAT CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG
1620 CGGCTGCATA CGCTTGATCC GGCTACCTGC CCATTGACC ACCAAGCGAA ACATCGCATC
1680 GAGCGAGCAC GTACTCGGAT GGAAGCCGGT CTTGTCGATC AGGATGATCT GGACGAAGAG
1740 CATCAGGGGC TCGCGCCAGC CGAACTGTTC GCCAGGCTCA AGGCGAGCAT GCCCGACGGC
1800 GAGGATCTCG TCGTGACCCA TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAAATGGC
1860 CGCTTTTCTG GATTCATCGA CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA
1920 GCGTTGGCTA CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCCTC
1980 GTGCTTTACG GTATCGCCGC TCCCGATTCTG CAGCGCATCG CCTTCTATCG CCTTCTTGAC
2040 GAGTTCTTCT GAGCGGGACT CTGGGGTTCG AAATGACCGA CCAAGCGACG CCCAACCTGC
2100 CATCACGAGA TTTCGATTCC ACCGCCGCCT TCTATGAAAG GTTGGGCTTC GGAATCGTTT
2160 TCCGGGACGC CGGCTGGATG ATCCTCCAGC GCGGGGATCT CATGCTGGAG TTCTTCGCCC
2220 ACCCTAGGGG GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGGA ACCCGCGCTA
2280 TGACGGCAAT AAAAAGACAG AATAAACGC ACGGTGTTGG GTCGTTTGTT CATAAACGCG
2340 GGGTTCGGTC CCAGGGCTGG CACTCTGTCTG ATACCCACCC GAGACCCCAT TGGGGCCAAT
2400 ACGCCCGCGT TTCTTCCTTT TCCCCACCCC ACCCCCCAAG TTCGGGTGAA GGCCAGGGC
2460 TCGCAGCCAA CGTCGGGGCG GCAGGCCCTG CCATAGCCTC AGGTTACTCA TATATACTTT
2520 AGATTGATTT AA-ACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA
2580 ATCTCATGAC CAAATCCCT TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG
2640 AAAAGATCAA AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAAA
2700 CAAAAAACC ACCGCTACCA GCGGTGGTTT GTTGCCGGA TCAAGAGCTA CCAACTCTTT
2760 TTCCGAAGGT AACTGGCTTC AGCAGAGCGC AGATACCAA TACTGTCCTT CTAGTGTAGC
2820 CGTAGTTAGG CCACCACTTC AAGAACTCTG TAGCACC GCC TACATACCTC GCTCTGCTAA
2880 TCCTGTTACC AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGGG TTGGACTCAA
2940 GACGATAGTT ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTCG TGCACACAGC
3000 CCAGCTTGGA GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA
3060 GCGCCACGCT TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA
3120 CAGGAGAGCG CACGAGGGAG CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCTG
3180

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FIG. 28. (CONTINUED)

GGTTTCGCCA CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC
3240
TATGGAAAAA CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG
3300
CTCACATGTT CTTTCCTGCG TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCATGC
3360
ATTAGTTATT AATAGTAATC AATTACGGGG TCATTAGTTC ATAGCCCATA TATGGAGTTC
3420
CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA CCCCCGCCCA
3480
TTGACGTCAA TAATGACGTA TGTTCCCATTA GTAACGCCAA TAGGGACTTT CCATTGACGT
3540
CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT GTATCATATG
3600
CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAATGGC CCGCCTGGCA TTATGCCCAG
3660
TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT
3720
ACCATGGTGA TGCGGTTTTG GCAGTACATC AATGGGCGTG GATAGCGGTT TGA CTCACGG
3780
GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAATCAA
3840
CGGGACTTTC CAAATGTCTG TAACAACTCC GCCCCATTGA CGCAAATGGG CGGTAGGCGT
3900
GTACGGTGGG AGGTCTATAT AAGCAGAGCT GGTTTAGTGA ACCGTCAGAT CCGCTAGCGC
3960
TACCGGTGCG CACCATGGTG AGCAAGGGCG AGGAGCTGTT CACCGGGGTG GTGCCCATCC
4020
TGGTCGAGCT GGACGGCGAC GTAAACGGCC ACAAGTTCAG CGTGTCCGGC GAGGGCGAGG
4080
GCGATGCCAC CTACGGCAAG CTGACCCTGA AGTTCATCTG CACCACCGGC AAGCTGCCCCG
4140
TGCCCTGGCC CACCCTCGTG ACCACCCTGA CCTACGGCGT GCAGTGCTTC AGCCGCTACC
4200
CCGACCACAT GAAGCAGCAC GACTTCTTCA AGTCCGCCAT GCCCGAAGGC TACGTCCAGG
4260
AGCGCACCAT CTTCTTCAAG GACGACGGCA ACTACAAGAC CCGCGCCGAG GTGAAGTTCG
4320
AGGGCGACAC CCTGGTGAAC CGCATCGAGC TGAAGGGCAT CGACTTCAAG GAGGACGGCA
4380
ACATCCTGGG GCACAAGCTG GAGTACAACT ACAACAGCCA CAACGTCTAT ATCATGGCCG
4440
ACAAGCAGAA GAACGGCATC AAGGTGAACT TCAAGATCCG CCACAACATC GAGGACGGCA
4500
GCGTGCAGCT CGCCGACCAC TACCAGCAGA ACACCCCAT CGGCGACGGC CCCGTGCTGC
4560
TGCCCGACAA CCACTACCTG AGCACCCAGT CCGCCCTGAG CAAAGACCCC AACGAGAAGC
4620
GCGATCACAT GGTCTGCTG GAGTTCGTGA CCGCCGCCGG GATCACTCTC GGCATGGACG
4680
AGCTGTACAA GTCCGGCCGG ACTCAGATCC CCATGAACCG TGCTTTTAGC AGGAAGAAAG
4740
ACAAAACATG GATGCATACA CCTGAAGCTT TATCAAAACA TTTCATTCCC TATAATGCAA
4800
AGTTTCTTGG CAGTACAGAA GTGGAACAGC CAAAAGGAAC AGAAGTTGTG AGAGATGCTG
4860

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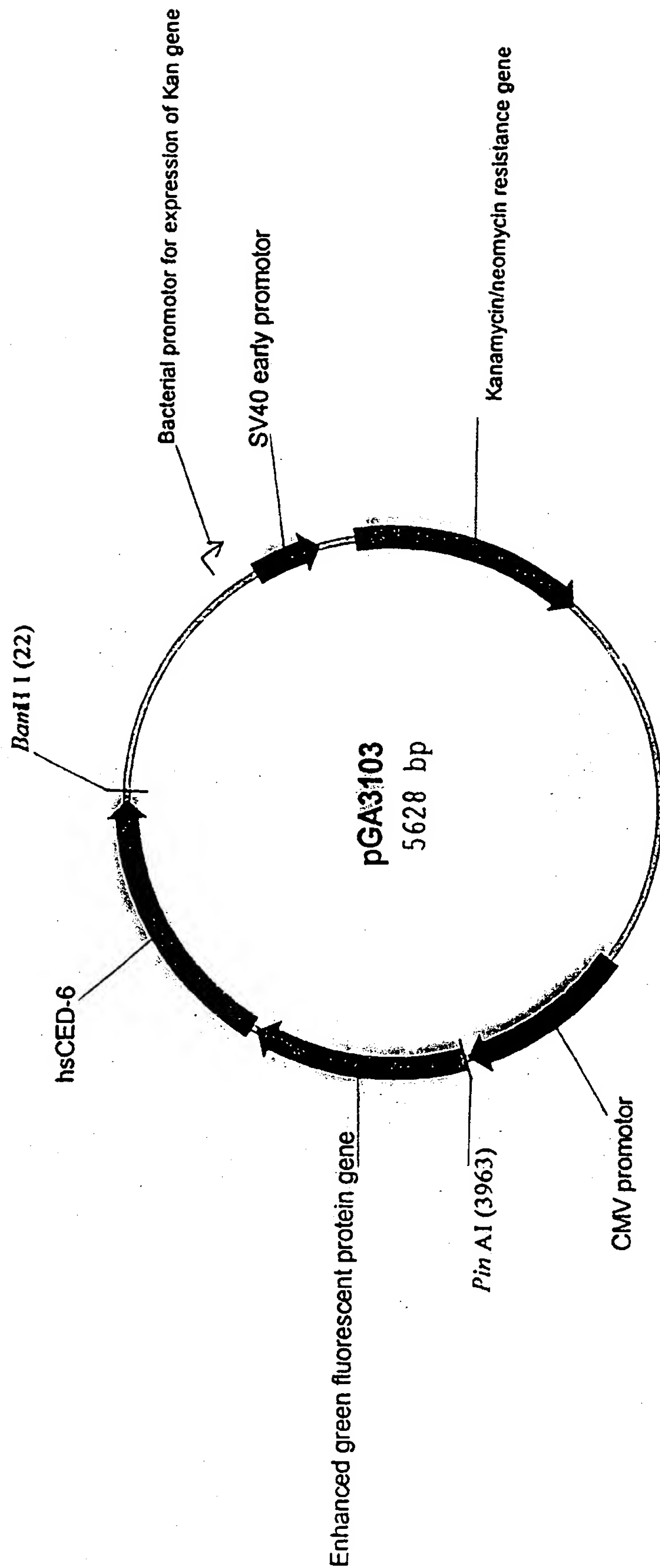
FIG. 28. (CONTINUED)

TAAGGAACT AAAGTTTGCA AGACATATCA AGAAATCTGA AGGCCAGAAA ATTCCTAAAG
4920 TGGAGTTGCA AATATCAATT TATGGAGTAA AAATTCTAGA ACCCAAACA AAGGAAGTTC
4980 AACACAATTG CCAGCTTCAT AGAATATCTT TTTGTGCAGA TGATAAACT GACAAGAGGA
5040 TATTCAC TTT CATATGCAAA GATTCTGAGT CAAATAAACA TTTGTGCTAT GTATTTGACA
5100 GCGAAAAGTG TGCTGAAGAG ATCACTTTAA CAATTGGCCA AGCATTTGAC CTGGCATACA
5160 CGAAATTTCT AGAATCAGGA GGAAAAGATG TTGAAACAAG AAAACAGATC GCAGGGTTAC
5220 AAAAAAGAAT CCAAGACTTA GAAACAGAAA ATATGGAAT TAAAAATAAA GTACAAGATT
5280 TGGAAAACCA ACTGAGAATA ACTCAAGTAT CAGCACCTCC AGCAGGCAGT ATGACACCTA
5340 AGTCGCCCTC CACTGACATC TTTGATATGA TTCCATTTTC TCCAATATCA CACCAGTCTT
5400 CGATGCCTAC TCGCAATGGC ACACAGCCAC CTCCAGTACC TAGTAGATCT ACTGAGATTA
5460 AACGGGACCT GTTTGGAGCA GAACCTTTTG ACCCATTTAA CTGTGGAGCA GCAGATTTCC
5520 CTCCAGATAT TCAATCAAAA TTAGATGAGA TGCAGGAGGG GTTCAAAATG GGACTAACTC
5580 TTGAAGGCAC AGTATTTTGT CTCGACCCGT TAGACAGTAG GTGCTGAG
5628

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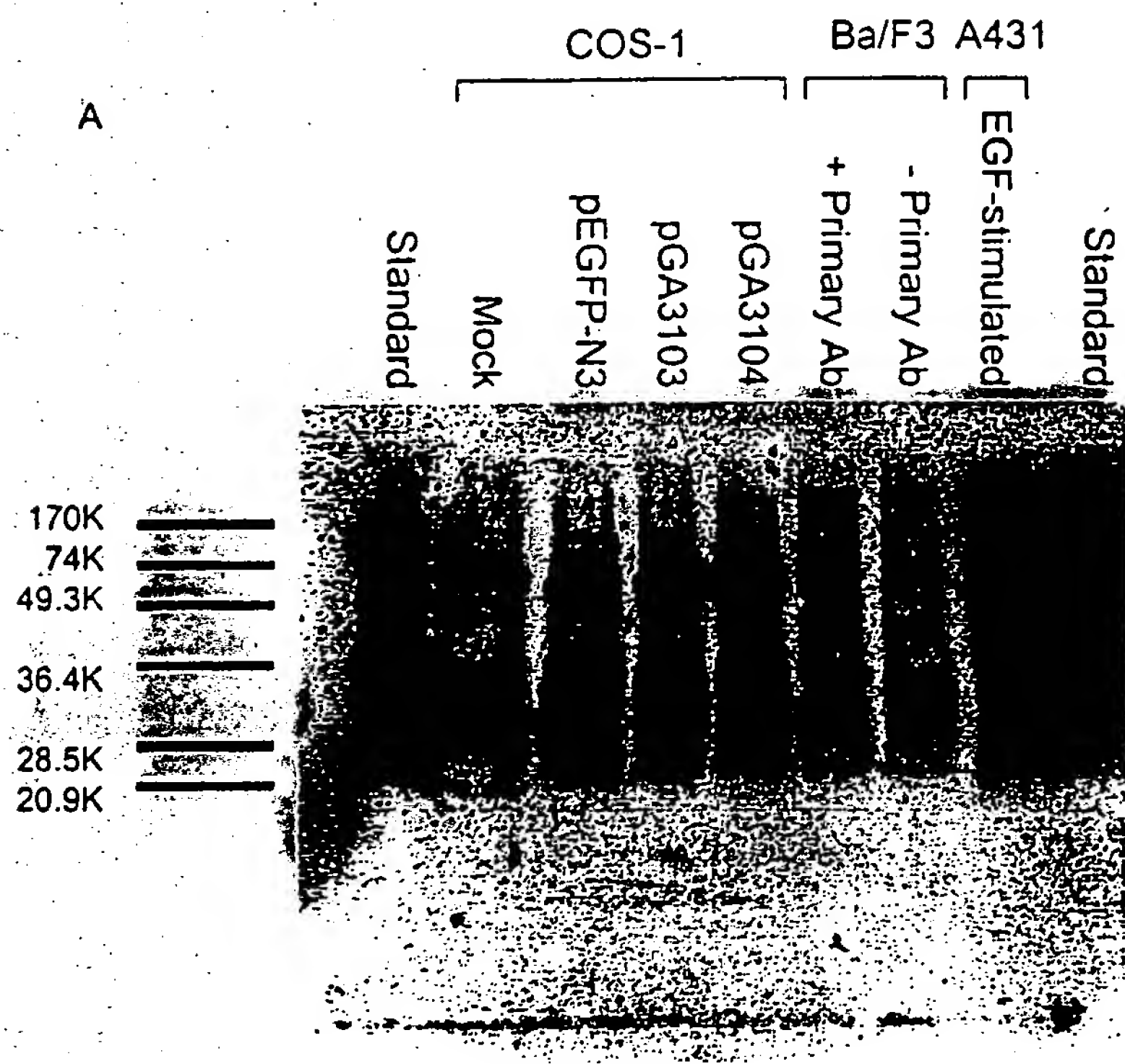
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FIG. 29.



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FIG. 30.



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FIG. 30. (CONTINUED)

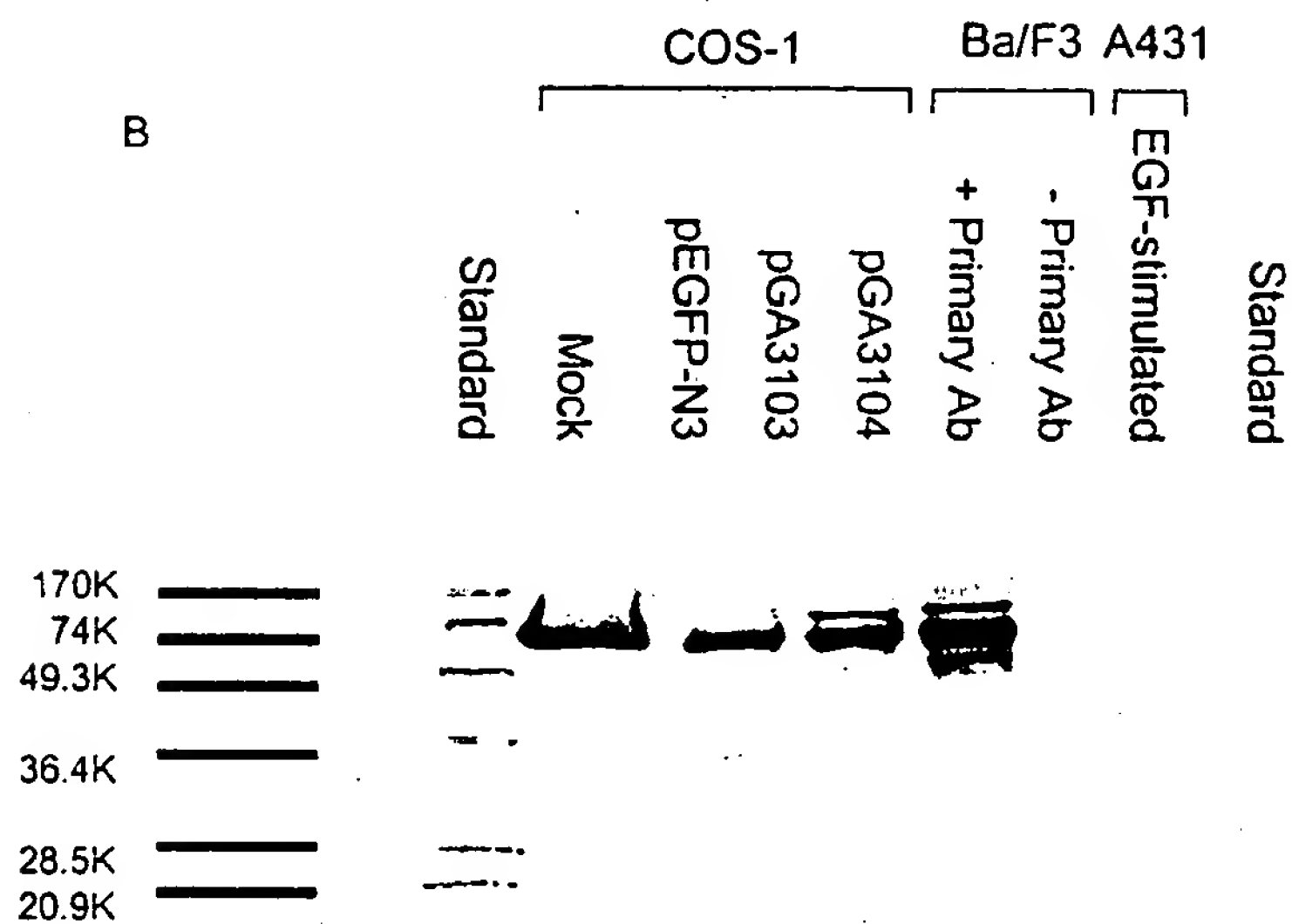
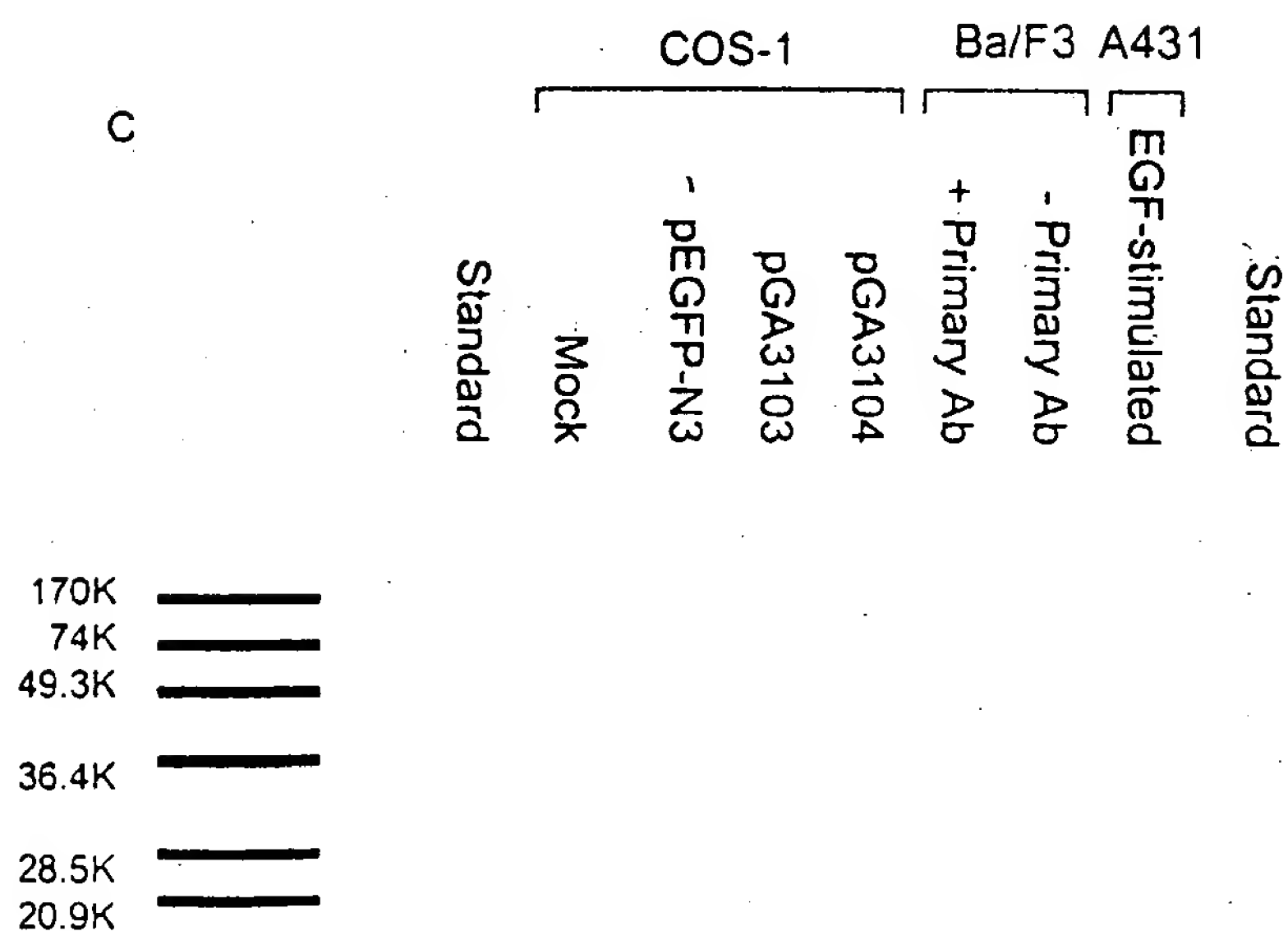


FIG. 30. (CONTINUED)



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FIG. 31

SQ SEQUENCE 6121 BP

60 GATCTATGGG CTGTGACCGG AACTGTGGGC TCATCGCTGG GGCTGTCATT GGTGCTGTCC
120 TGGCTGTGTT TGGAGGTATT CTAATGCCAG TTGGAGACCT GCTTATCCAG AAGACAATTA
180 AAAAGCAAGT TGTCTCGAA GAAGGTACAA TTGCTTTTAA AAATTGGGTT AAAACAGGCA
240 CAGAAGTTTA CAGACAGTTT TGGATCTTTG ATGTGCAAAA TCCACAGGAA GTGATGATGA
300 ACAGCAGCAA CATTCAAGTT AAGCAAAGAG GTCCTTATAC GTACAGAGTT CGTTTTCTAG
360 CCAAGGAAAA TGTAACCCAG GACGCTGAGG ACAACACAGT CTCTTTCCTG CAGCCCAATG
420 GTGCCATCTT CGAACCTTCA CTATCAGTTG GAACAGAGGC TGACAACTTC ACAGTTCTCA
480 ATCTGGCTGT GGCAGCTGCA TCCCATATCT ATCAAAATCA ATTTGTTCAA ATGATCCTCA
540 ATTCACTTAT TAACAAGTCA AAATCTTCTA TGTTCOAAGT CAGAACTTTG AGAGAACTGT
600 TATGGGGCTA TAGGGATCCA TTTTGTAGTT TGGTCCGTA CCCTGTTACT ACTACAGTTG
660 GTCTGTTTTA TCCTTACAAC AATACTGCAG ATGGAGTTTA TAAAGTTTTT AATGGAAAAG
720 ATAACATAAG TAAAGTTGCC ATAATCGACA CATATAAAGG TAAAAGGAAT CTGTCCTATT
780 GGGAAAGTCA CTGCGACATG ATTAATGGTA CAGATGCAGC CTCATTTCOA CCTTTTGTG
840 AGAAAAGCCA GGTATTGCAG TTCTTTTCTT CTGATATTG CAGGTCAATC TATGCTGTAT
900 TTGAATCCGA CGTTAATCTG AAAGGAATCC CTGTGTATAG ATTCGTTCTT CCATCCAAGG
960 CCTTTGCCTC TCCAGTTGAA AACCCAGACA ACTATTGTTT CTGCACAGAA AAAATTATCT
1020 CAAAAAATTG TACATCATAT GGTGTGCTAG ACATCAGCAA ATGCAAAGAA GGGAGACCTG
1080 TGTACATTTT ACTTCCTCAT TTTCTGTATG CAAGTCCTGA TGTTTCAGAA CCTATTGATG
1140 GATTAAACCC AAATGAAGAA GAACATAGGA CATACTTGA TATTCAACCT ATAAGTGGAT
1200 TCACTTTACA ATTTGCAAAA CGGCTGCAGG TCAACCTATT GGTCAAGCCA TCAGAAAAAA
1260 TTCAAGTATT AAAGAATCTG AAGAGGAACT ATATTGTGCC TATTCTTTGG CTTAATGAGA
1320 CTGGGACCAT TGGTGATGAG AAGGCAAACA TGTTCAGAAG TCAAGTAACT GGAAAAATAA
1380 ACCTCCTTGG CCTGATAGAA ATGATCTTAC TCAGTGTTGG TGTGGTGATG TTTGTTGCTT
1440 TTATGATTTT ATATTGTGCA TGCAGATCGA AAACAATAAA AGTCGACGGT ACCGCGGGCC
1500 CGGGATCCAT CGCCACCATG GTGAGCAAGG GCGAGGAGCT GTTCACCGGG GTGGTGCCCA

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FIG. 31. (CONTINUED)

TCCTGGTCGA GCTGGACGGC GACGTAAACG GCCACAAGTT CAGCGTGTCC GGCGAGGGCG
1560
AGGGCGATGC CACCTACGGC AAGCTGACCC TGAAGTTCAT CTGCACCACC GGCAAGCTGC
1620
CCGTGCCCTG GCCCACCCTC GTGACCACCC TGACCTACGG CGTGCAGTGC TTCAGCCGCT
1680
ACCCCGACCA CATGAAGCAG CACGACTTCT TCAAGTCCGC CATGCCCCGAA GGCTACGTCC
1740
AGGAGCGCAC CATCTTCTTC AAGGACGACG GCAACTACAA GACCCGCGCC GAGGTGAAGT
1800
TCGAGGGCGA CACCCTGGTG AACCGCATCG AGCTGAAGGG CATCGACTTC AAGGAGGACG
1860
GCAACATCCT GGGGCACAAG CTGGAGTACA ACTACAACAG CCACAACGTC TATATCATGG
1920
CCGACAAGCA GAAGAACGGC ATCAAGGTGA ACTTCAAGAT CCGCCACAAC ATCGAGGACG
1980
GCAGCGTGCA GCTCGCCGAC CACTACCAGC AGAACACCCC CATCGGCGAC GGCCCCGTGC
2040
TGCTGCCCGA CAACCACTAC CTGAGCACCC AGTCCGCCCT GAGCAAAGAC CCCAACGAGA
2100
AGCGCGATCA CATGGTCCTG CTGGAGTTCG TGACCGCCGC CGGGATCACT CTCGGCATGG
2160
ACGAGCTGTA CAAGTAAAGC GGCCGCGACT CTAGATCATA ATCAGCCATA CCACATTTGT
2220
AGAGGTTTTA CTTGCTTTAA AAAACCTCCC ACACCTCCCC CTGAACCTGA AACATAAAAT
2280
GAATGCAATT GTTGTGTGTA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA
2340
TAGCATCACA AATTTACAA ATAAAGCATT TTTTTCCTG CATTCTAGTT GTGGTTTGTC
2400
CAAATCATC AATGTATCTT AAGGCGTAAA TTGTAAGCGT TAATATTTTG TTAAAATTCTG
2460
CGTTAAATTT TTGTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC GGCAAAATCC
2520
CTTATAAATC AAAAGAATAG ACCGAGATAG GGTGAGTGT TGTTCCAGTT TGGAACAAGA
2580
GTCCACTATT AAAGAACGTG GACTCCAACG TCAAAGGGCG AAAAACCGTC TATCAGGGCG
2640
ATGGCCCACT ACGTGAACCA TCACCCTAAT CAAGTTTTTT GGGGTCGAGG TGCCGTAAAG
2700
CACTAAATCG GAACCCTAAA GGGAGCCCCC GATTTAGAGC TTGACGGGGA AAGCCGGCGA
2760
ACGTGGCGAG AAAGGAAGGG AAGAAAGCGA AAGGAGCGGG CGCTAGGGCG CTGGCAAGTG
2820
TAGCGGTCAC GCTGCGCGTA ACCACCACAC CCGCCGCGCT TAATGCGCCG CTACAGGGCG
2880
CGTCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTCTAAA
2940
TACATTCAA TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT CAATAATATT
3000
GAAAAAGGAA GAGTCCTGAG GCGGAAAGAA CCAGCTGTGG AATGTGTGTC AGTTAGGGTG
3060
TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC
3120
AGCAACCAGG TGTGGAAAGT CCCCAGGCTC CCCAGCAGGC AGAAGTATGC AAAGCATGCA
3180

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FIG. 31. (CONTINUED)

TCTCAATTAG TCAGCAACCA TAGTCCCGCC CCTAACTCCG CCCATCCCGC CCCTAACTCC
3240
GCCCAGTTCC GCCCATTCTC CGCCCCATGG CTGACTAATT TTTTTTATTT ATGCAGAGGC
3300
CGAGGCCGCC TCGGCCTCTG AGCTATTCCA GAAGTAGTGA GGAGGCTTTT TTGGAGGCCT
3360
AGGCTTTTGC AAAGATCGAT CAAGAGACAG GATGAGGATC GTTTCGCATG ATTGAACAAG
3420
ATGGATTGCA CGCAGGTTCT CCGGCCGCTT GGGTGGAGAG GCTATTCGGC TATGACTGGG
3480
CACAACAGAC AATCGGCTGC TCTGATGCCG CCGTGTTCCG GCTGTCAGCG CAGGGGCGCC
3540
CGGTTCTTTT TGTCAAGACC GACCTGTCCG GTGCCCTGAA TGAAGTCAA GACGAGGCAG
3600
CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC AGCTGTGCTC GACGTTGTCA
3660
CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC GGGGCAGGAT CTCCTGTCAT
3720
CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA TGCAATGCGG CGGCTGCATA
3780
CGCTTGATCC GGCTACCTGC CCATTCGACC ACCAAGCGAA ACATCGCATC GAGCGAGCAC
3840
GTAATCGGAT GGAAGCCGGT CTTGTGATC AGGATGATCT GGACGAAGAG CATCAGGGGC
3900
TCGCGCCAGC CGAACTGTTC GCCAGGCTCA AGGCGAGCAT GCCCGACGGC GAGGATCTCG
3960
TCGTGACCCA TGGCGATGCC TGCTTGCCGA ATATCATGGT GGAAAATGGC CGCTTTTCTG
4020
GATTCATCGA CTGTGGCCGG CTGGGTGTGG CGGACCGCTA TCAGGACATA GCGTTGGCTA
4080
CCCGTGATAT TGCTGAAGAG CTTGGCGGCG AATGGGCTGA CCGCTTCCTC GTGCTTTACG
4140
GTATCGCCGC TCCCGATTCC CAGCGCATCG CCTTCTATCG CCTTCTTGAC GAGTTCTTCT
4200
GAGCGGGACT CTGGGGTTCG AAATGACCGA CCAAGCGACG CCCAACCTGC CATCACGAGA
4260
TTTCGATTCC ACCGCCGCCT TCTATGAAAG GTTGGGCTTC GGAATCGTTT TCCGGGACGC
4320
CGGCTGGATG ATCCTCCAGC GCGGGGATCT CATGCTGGAG TTCTTCGCCC ACCCTAGGGG
4380
GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGGA ACCCGCGCTA TGACGGCAAT
4440
AAAAAGACAG AATAAAACGC ACGGTGTTGG GTCGTTTGTT CATAAACGCG GGGTTCGGTC
4500
CCAGGGCTGG CACTCTGTGC ATACCCACCC GAGACCCCAT TGGGGCCAAT ACGCCCGCGT
4560
TTCTTCCTTT TCCCCACCCC ACCCCCCAAG TTCGGGTGAA GGCCAGGGC TCGCAGCCAA
4620
CGTCGGGGCG GCAGGCCCTG CCATAGCCTC AGGTTACTCA TATATACTTT AGATTGATTT
4680
AAACTTCAT TTTTAATTTA AAAGGATCTA GGTGAAGATC CTTTTTGATA ATCTCATGAC
4740
CAAAATCCCT TAACGTGAGT TTTCGTTCCA CTGAGCGTCA GACCCCGTAG AAAAGATCAA
4800
AGGATCTTCT TGAGATCCTT TTTTCTGCG CGTAATCTGC TGCTTGCAA CAAAAAACC
4860

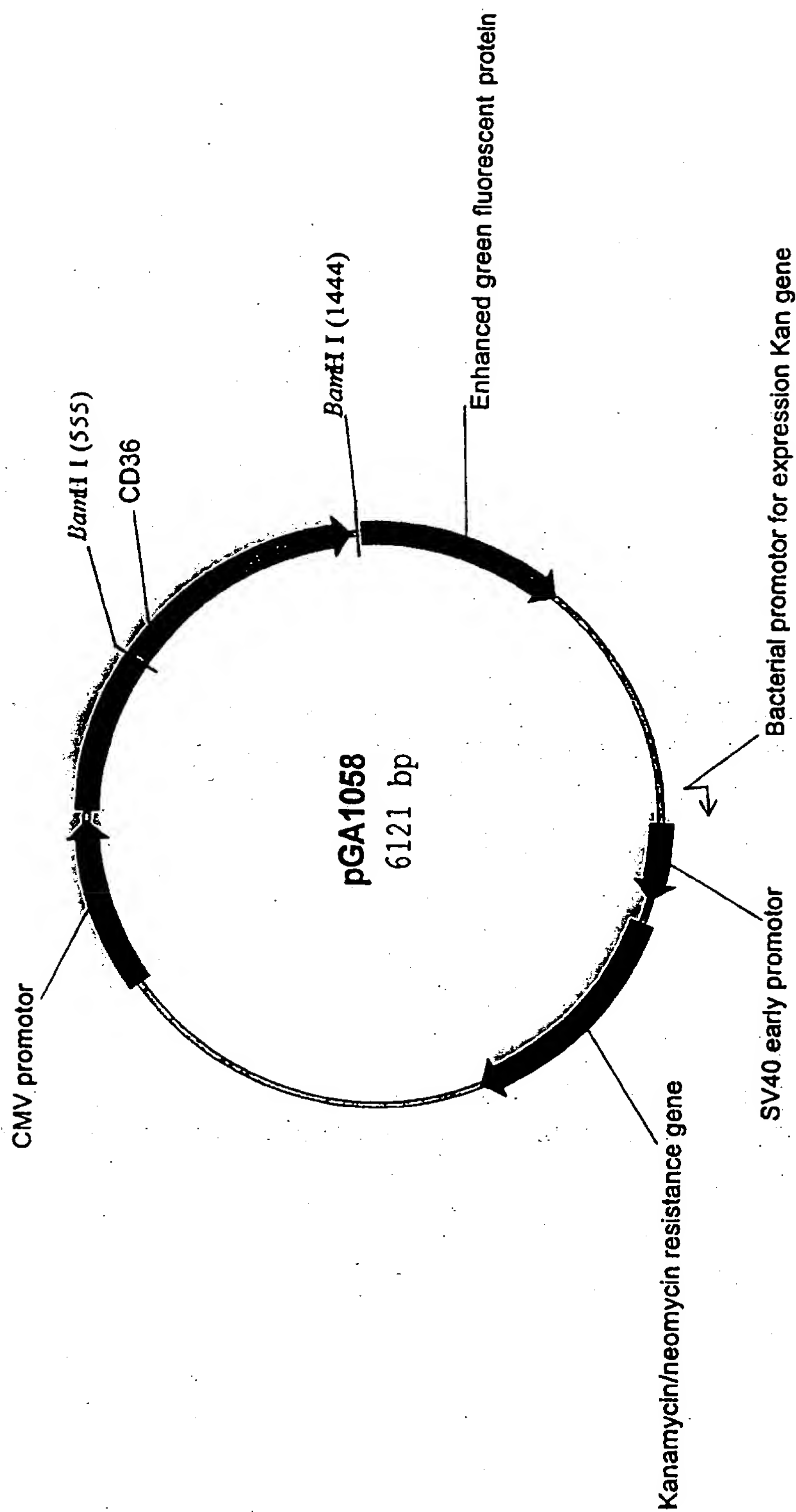
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FIG. 31. (CONTINUED)

ACCGCTACCA GCGGTGGTTT GTTTGCCGGA TCAAGAGCTA CCAACTCTTT TTCCGAAGGT
4920
AACTGGCTTC AGCAGAGCGC AGATACCAA TACTGTCCTT CTAGTGTAGC CGTAGTTAGG
4980
CCACCACTTC AAGAACTCTG TAGCACC GCC TACATACCTC GCTCTGCTAA TCCTGTTACC
5040
AGTGGCTGCT GCCAGTGGCG ATAAGTCGTG TCTTACCGG TTGGACTCAA GACGATAGTT
5100
ACCGGATAAG GCGCAGCGGT CGGGCTGAAC GGGGGGTTTCG TGCACACAGC CCAGCTTGGA
5160
GCGAACGACC TACACCGAAC TGAGATACCT ACAGCGTGAG CTATGAGAAA GCGCCACGCT
5220
TCCCGAAGGG AGAAAGGCGG ACAGGTATCC GGTAAGCGGC AGGGTCGGAA CAGGAGAGCG
5280
CACGAGGGAG CTTCCAGGGG GAAACGCCTG GTATCTTTAT AGTCCTGTCG GGTTTCGCCA
5340
CCTCTGACTT GAGCGTCGAT TTTTGTGATG CTCGTCAGGG GGGCGGAGCC TATGGAAAAA
5400
CGCCAGCAAC GCGGCCTTTT TACGGTTCCT GGCCTTTTGC TGGCCTTTTG CTCACATGTT
5460
CTTTCCTGCG TTATCCCCTG ATTCTGTGGA TAACCGTATT ACCGCCATGC ATTAGTTATT
5520
AATAGTAATC AATTACGGGG TCATTAGTTC ATAGCCCATATA TATGGAGTTC CGCGTTACAT
5580
AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA CCCCCGCCCA TTGACGTCAA
5640
TAATGACGTA TGTTCACATA GTAACGCCAA TAGGGACTTT CCATTGACGT CAATGGGTGG
5700
AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT GTATCATATG CCAAGTACGC
5760
CCCCTATTGA CGTCAATGAC GGTAAATGGC CCGCCTGGCA TTATGCCCAG TACATGACCT
5820
TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT ACCATGGTGA
5880
TGCGGTTTTG GCAGTACATC AATGGGCGTG GATAGCGGTT TGA CTCACGG GGATTTCCAA
5940
GTCTCCACCC CATTGACGTC AATGGGAGTT TGT TTTGGCA CCAAATCAA CGGGACTTTC
6000
CAAATGTCG TAACAACTCC GCCCATTTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG
6060
AGGTCTATAT AAGCAGAGCT GGT TTAGTGA ACCGTCAGAT CCGCTAGCGC TACCGGACTC
6120
A
6121
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FIG. 32.



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FIG. 33.

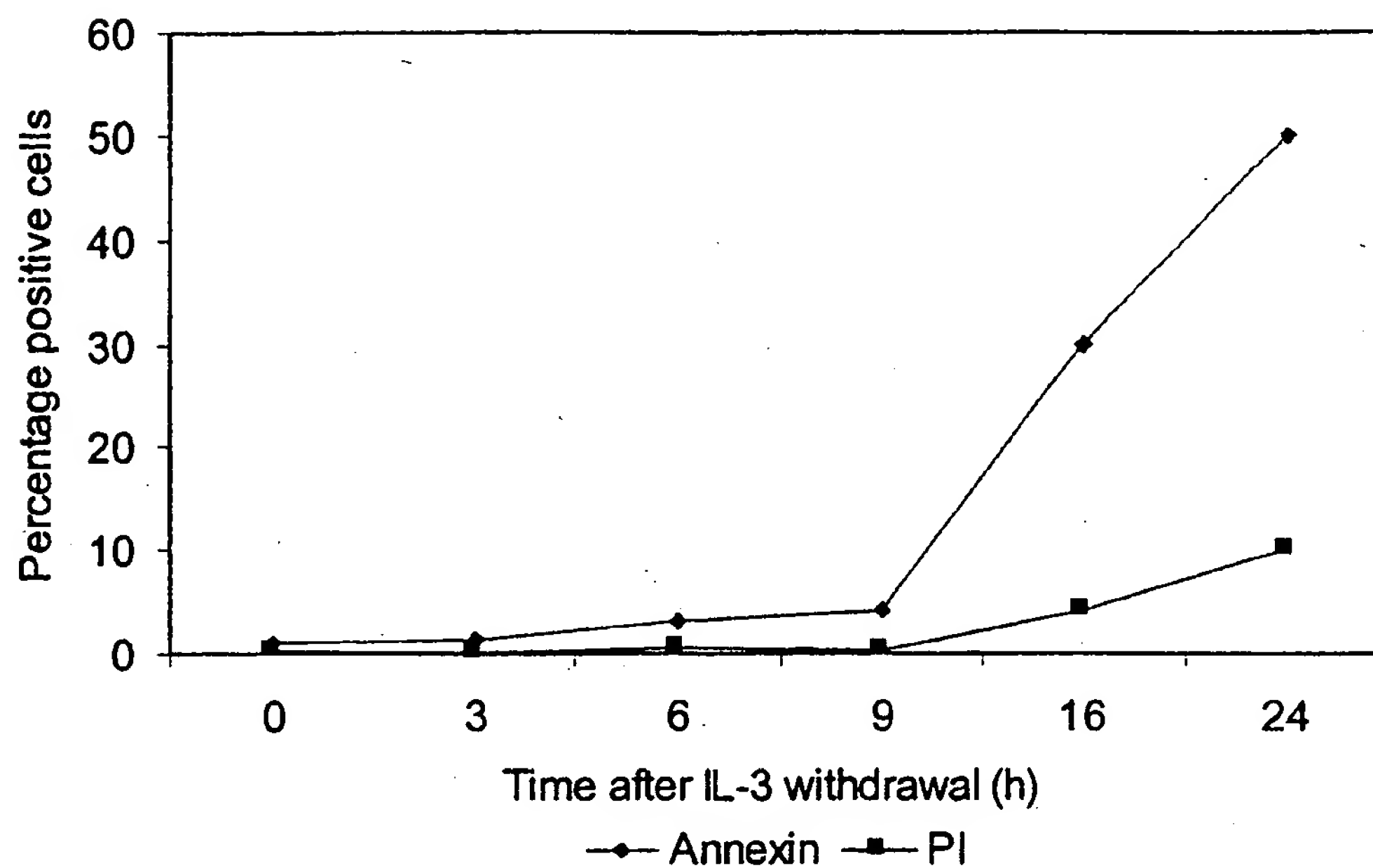
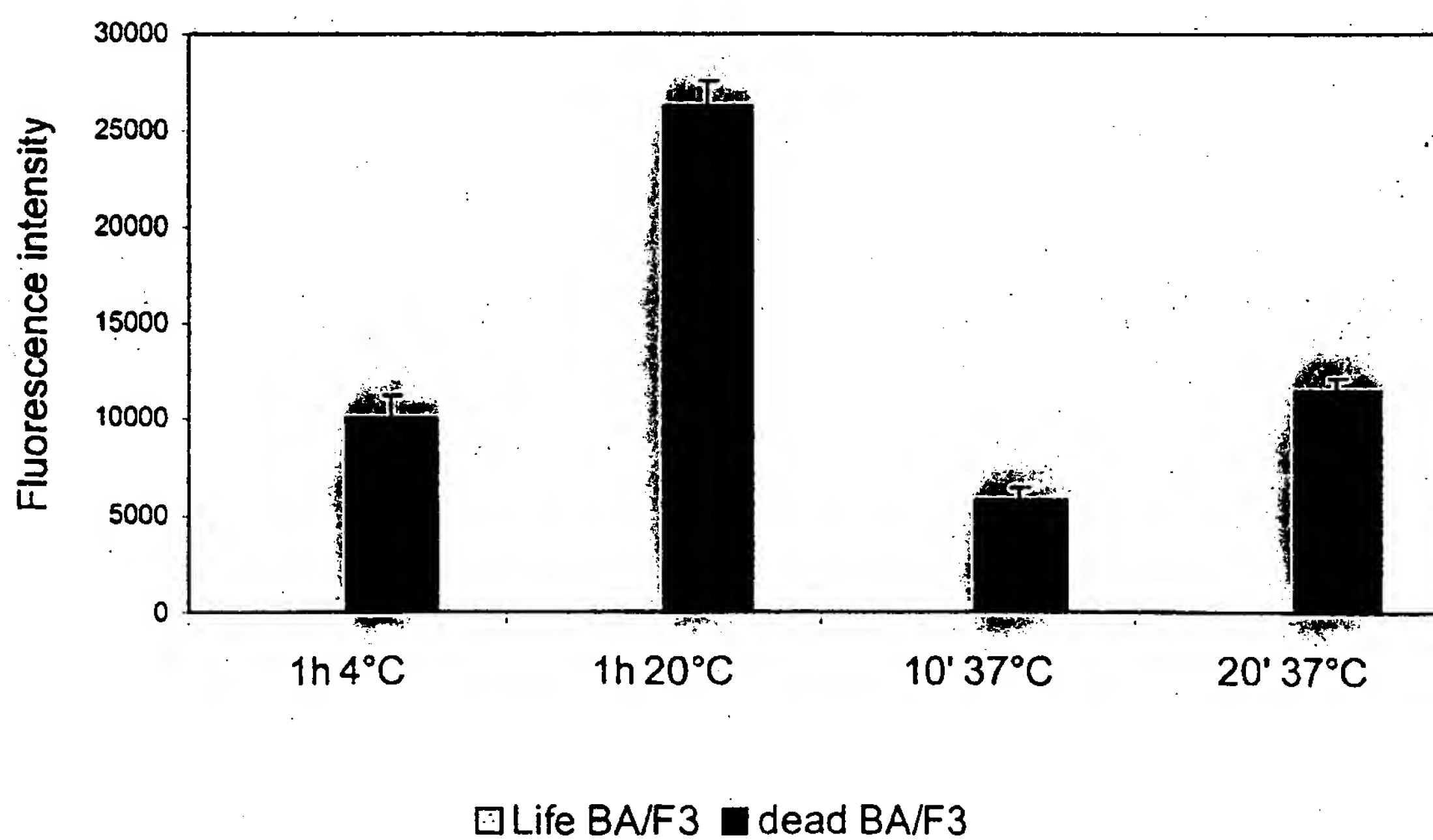
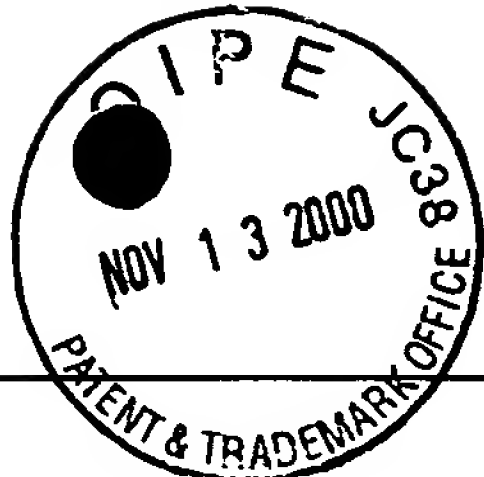


FIG. 34.





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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.			
Typed or Printed Name	Donna Macedo.		
Signature	<i>Df Macedo</i>	Date	11/7/2000
SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. § 1.97(e) Address to: Assistant Commissioner for Patents Washington, D.C. 20231		Attorney Docket	TOSK-004
		First Named Inventor	FOGARTY, Patrick
		Application Number	09/472,654
		Filing Date	December 27, 1999
		Group Art Unit	1648
		Examiner Name	Unassigned
		Title	<i>In Vivo High Throughput Toxicology Screening Method</i>

Sir:

Applicants submit herewith patents and/or publications which may be material to the examination of this application and in respect of which there may be a duty to disclose in accordance with 37 C.F.R. §1.56. While this Statement may be "material" pursuant to 37 C.F.R. §1.56, it is not intended to constitute an admission that any patent, publication, or other information referred to therein is "prior art" for this invention unless specifically designated as such. A listing of patents and/or publications is shown on enclosed Form PTO-1449 and a copy of each patent and/or publication is also enclosed.

Each item of information contained in the Information Disclosure Statement filed herewith was cited in a communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this Statement (37 C.F.R. 1.97(e)(1)). A copy of the communication is enclosed for the Examiner's convenience.

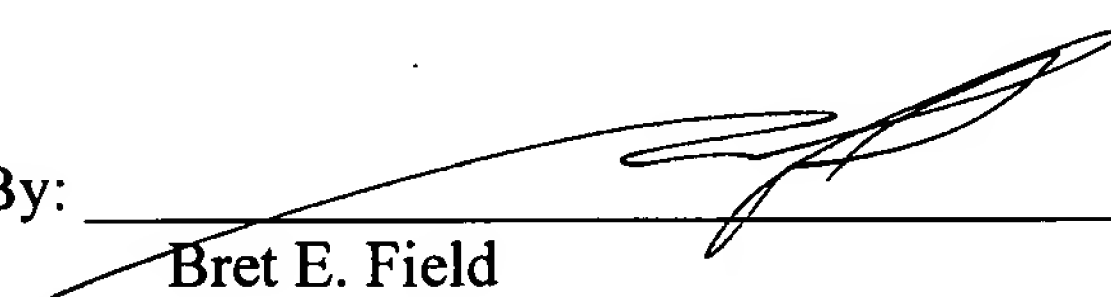
The Examiner is requested to make the citations listed on the enclosed PTO 1449 of record in this application. Applicants would appreciate the Examiner initialing and returning the initialed copy of form PTO 1449, indicating the references have been considered and made of record herein.

Atty Dkt. No.: TOSK-004
USSN: 09/472,654

No fee is believed due as this statement is being submitted within three months of the mailing date of the enclosed foreign communication. However, if it is determined that fees are required, the Commissioner is hereby authorized to charge any necessary fees associated with this communication or credit any overpayment to Deposit Account No. 50-0815.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: 11.7.00

By: 
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